JOURNAL OF AGRICULTURAL RESEARCH

VOL. 59 WASHINGTON, D. C., JULY 15, 1939 No. 2

EFFECT OF VARIOUS GRADES OF FERTILIZERS ON THE SALT CONTENT OF THE SOIL SOLUTION [1]

By LAWRENCE M. WHITE, *assistant chemist*, and WILLIAM H. ROSS, *senior chemist, Fertilizer Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture* [2]

INTRODUCTION

Crops grown on different soils differ greatly not only in yields but also in quality, appearance, and resistance to spoilage. Climate, water relations, biological conditions, and the physical properties of a soil have a great deal to do with its crop-producing capacity, but even with these factors at the optimum good crops cannot be obtained if the soil solution contains an excess of soluble salts or an inadequate supply of any essential plant-food element.

One of the most effective methods for controlling the composition of the soil solution is treatment with fertilizers. Soluble salts of some of the minor fertilizer elements are toxic to plants at relatively low concentrations, injuring them by changing the permeability of their cell walls or otherwise interfering with their metabolism. The ordinary fertilizer salts are less toxic to plants and give rise to crop burning only when a sufficient quantity is present to develop an osmotic pressure in the soil solution that approaches or exceeds that of the plant sap. Water then enters the plant too slowly to compensate for that lost by transpiration, or it actually passes from the roots by osmosis. In either case, the plant withers and dies.

The purpose of this investigation was to develop a laboratory method for measuring the influence of fertilizers on the concentration of the soil solution with a view to determining whether danger from salt injury is being increased or decreased by the changes taking place in the composition of fertilizers. The work was limited to a study of the ordinary fertilizer materials and mixtures which are considered to be nontoxic to plants below a concentration that produces plasmolysis in the ordinary cultivated crops. It is recognized that factors other than concentration determine the burning effects of solutions. Composition, especially the relative proportion of divalent and monovalent elements, is of particular importance. The solutions recovered in this work from soils treated with various fertilizer materials contained calcium as well as the monovalent elements resulting from base-exchange reactions. With one exception both types of elements were present in all mixtures used in the tests.

PROCEDURE

Some fertilizer materials react with the constituents of the soil to a much greater extent than others. A mixture containing a high pro-

[1] Received for publication March 13, 1939.

[2] The authors acknowledge their indebtedness to H. G. Byers and M. S. Anderson, Soil Chemistry and Physics Research Division, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful suggestions in carrying out this investigation; and to Charles E. Kellogg, Chief, Soil Survey Division, Bureau of Plant Industry, for supplying the soils used in the experimental work.

portion of soluble salts that undergo fixation in the soil may therefore increase the concentration of the soil solution less than one containing a lower percentage of soluble salts that are not fixed in the soil. The soluble-salt content of different fertilizer mixtures cannot therefore be employed as an accurate measure of their influence on the soil solution. The method used in this investigation for determining the effect of any given fertilizer consisted in making a direct determination of the change in the effective concentration of the soil solution as a result of the fertilizer treatment.

Methods proposed for obtaining the true soil solution may be grouped into three classes, depending on whether the solution is separated from the soil by (1) application of high pressure (*8*)[3]; (2) centrifugal action (*2*); or (3) displacement with a liquid (*3*, *4*, *6*, *9*, *10*, *14*, *15*).

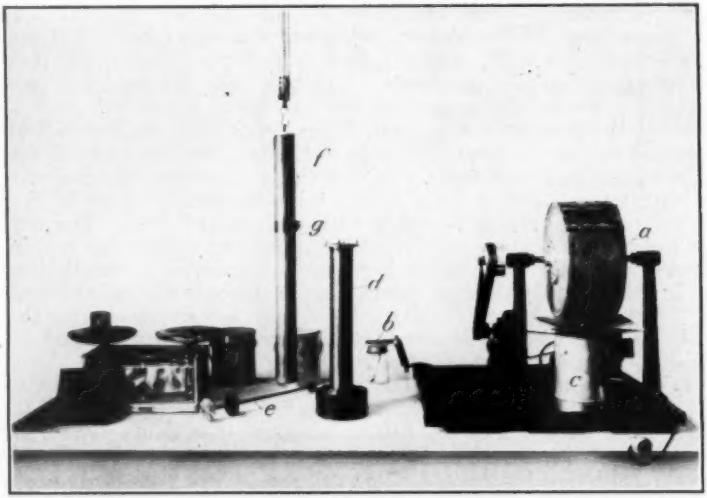

FIGURE 1.—Equipment for treating the soil preparatory to the recovery of the soil solution.

The displacement method has advantages over the other two methods in that it requires less complicated apparatus and permits the recovery of larger quantities of the soil solution from a soil of given moisture content. It was first developed by Schlœsing (*14*), who used water colored with carmine to displace the soil solution. Ishcherekov (*6*) used ethyl alcohol as the displacing agent and obtained results which indicated that the displaced solution is the true soil solution. Parker (*10*) showed that the use of such different displacing agents as acetone, methyl alcohol, water, and ethyl alcohol has little or no effect on the concentration of the recovered soil solution and that the displacement method is well adapted to "a study of the composition and reaction of the soil solution under any condition."

That the composition of the solution recovered by displacement approximates that of the true soil solution was further demonstrated

[3] Italic numbers in parentheses refer to Literature Cited, p. 98.

by Burd and Martin (*3*), who used the displaced soil solution from a portion of soil as the displacing medium for a second portion of the same soil. The newly displaced solution had the same concentration as the displacing solution, indicating that this displaced solution had the same concentration as the solution with which it came in contact in the soil. It was also shown by Burd and Martin that the time of recovering the displaced solution could be shortened and the yield of solution by water displacement increased by applying air pressure to the closely packed soil in a closed container. Accordingly, the displacement method was adopted for the recovery of the soil solution in this investigation.

The fertilizer to be tested was mixed with the soil in an air-dried condition. The soil was then sprayed with sufficient water to bring

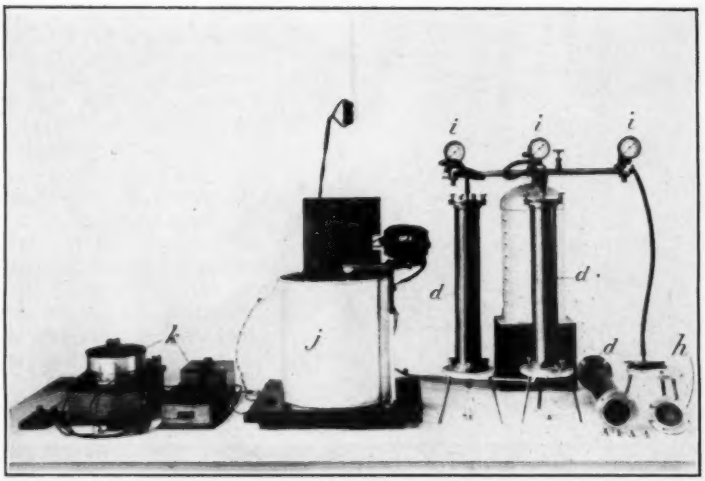

FIGURE 2.—Equipment for displacing the soil solution and measuring its specific conductivity.

the moisture content to 75 percent of its moisture equivalent (*1*, *2*), after which it was stored in a closed container until it reached a state of equilibrium with respect to the added moisture and fertilizer. A comparison of the concentration of soil solutions from samples stored for 1, 3, 5, and 30 days showed that storage for 5 days is ample for this purpose. In most of the tests the samples were maintained at 5° C. during storage with a view to avoiding appreciable change in the composition of the soil solution as a result of biological action. The soil solution was finally displaced from the soil, and the effect of the added fertilizer was determined by comparing the concentration of the recovered solution with that of the soil solution displaced from the unfertilized soil.

The equipment used in the work is shown in figures 1 and 2.

Figure 1 shows a device (*a*) for mixing the fertilizer with the dry soil, a spray gun (*b*) for spraying the water on the soil while it is being rolled on a rubber sheet, friction-top cans (*c*) for storing the soil, and equip-

ment for packing the stored soil into the displacement cylinders (*d*). The soil was transferred to the cylinders in increments of 150 gm. The surface of the soil in the cylinder was leveled off with the instrument (*e*), and each addition of soil was then packed in the cylinder by allowing the plunger (*f*) to fall three times from a height of 12 cm., as measured by the chain (*g*). The soils were packed to a degree that permitted ready flow of the displaced solution without excessive dilution by the displacing medium because of too rapid flow or channeling. A 40-pound plunger gave the best results with the Norfolk sandy loam soil used in the tests and a 20-pound plunger with the Cecil clay loam soil.

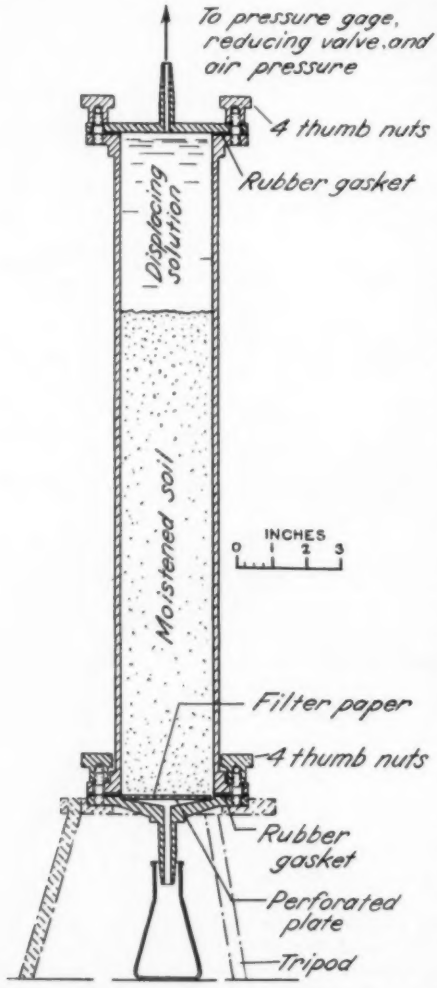

FIGURE 3.—Brass cylinder for displacing soil solutions.

Figure 2 shows the reducing valves and gages (*i*) for controlling and measuring the pressure applied to each cylinder, the constant temperature bath (*j*) in which cells *C* and *D* (fig. 4) were supported to bring the solution to the desired temperature (30° C.) for measurement, and the electrical equipment (*k*) used in making the conductivity measurements.

Each cylinder, shown assembled in figure 2 and diagrammatically in figure 3, was 7 cm. in diameter and 45 cm. long, constructed of brass throughout, and provided with a perforated bottom (fig. 2, *h*), over which a filter paper was placed to support the soil.

The liquid used as displacing agent was poured over the soil in the cylinder to a depth of about 12 cm. The top of the cylinder was then closed, and after 1 hour sufficient air pressure was applied to cause the displaced soil solution to drop rapidly from the cylinder. A clear solution was recovered from both soils used in the tests except

when treated with excessive applications of fertilizers or with a basic material such as free ammonia.

Distilled water was used as the displacing agent for soils receiving a large application of fertilizer, while 0.1 normal sodium chloride solution was used as the displacing agent for soils having a low content of soluble salts. In this way a marked difference was always maintained between the concentration of the soil solution and that of the displacing agent, and the point at which further recovery of the solution would mean contamination with the displacing agent could be readily determined by making conductivity determinations of successive 5- or 10-cc. portions of the recovered solution.

The types of conductivity cells used in the work are shown in figure 4. Conductivity cells *A* and *B* were used in making quick tests

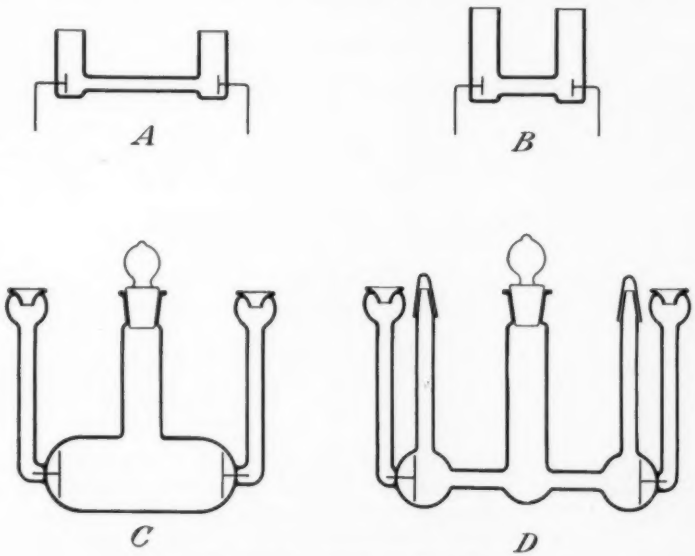

FIGURE 4.—Conductivity cells.

for any change in the concentration of the recovered soil solution, while cells *C* and *D* were used in measuring the relative concentrations of different soil solutions by a determination of their specific conductivities. Conductivity cell *C* was adapted for measuring the concentration of solutions having a specific conductivity below 2×10^{-3} ohm^{-1}, while cell *D* was used for solutions having a specific conductivity in excess of this value. The constants of cells *C* and *D* were determined by the method of Parker and Parker (*11*).

Specific conductivity measurements are adapted for determining the relative concentrations of solutions containing the same or similar combinations of inorganic salts, but freezing-point measurements give more accurate results for the effective concentrations of solutions that contain organic materials. As a check on the results both methods were used for all solutions, but the results with both methods are

shown only in figure 12. In all the other figures in which curves are shown the variation in the effective concentration of the soil solution with increased application of a fertilizer is expressed in terms of its osmotic pressure, as calculated from the freezing-point depression.

The osmotic pressure P, in atmospheres, of an aqueous solution at its freezing point may be calculated (7) from the difference Δ, in degrees centigrade, between its freezing point and that of pure water by the equation

$$P=12.06\Delta-0.021\Delta^2 \quad (1)$$

Values of P in this equation may be conveniently obtained from the table of Harris and Gortner (5) for all values of Δ between 0.001° and 2.999° C. All freezing-point determinations were made by the Beckmann method, which has an accuracy of $\pm 0.005°$ (3).

SOILS USED IN TESTS

Two soils were used in the tests: A Norfolk sandy loam of low fixing power from Edgefield County, S. C., and a Cecil clay loam of high fixing power from Abbeville County, S. C. Before it was used, each soil was air-dried and passed through a 10-mesh screen. The mechanical analyses of the two soils are given in table 1.

TABLE 1.—*Mechanical analysis of soils* [1]

Type of soil	Fine gravel	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Clay	Colloid (included in clay)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Norfolk sandy loam	6.0	25.2	15.8	24.8	13.2	8.5	6.3	4.2
Cecil clay loam	1.3	3.3	3.9	16.1	11.7	16.2	47.2	41.3

[1] Analysis by T. M. Shaw, Soil Chemistry and Physics Research Division, Bureau of Plant Industry.

The pH value of the Norfolk soil used in this investigation was 5.29 as determined by the hydrogen electrode, and that of the Cecil soil was 5.20. The Norfolk soil had a moisture equivalent of 5.1 percent; that of the Cecil soil was 26.3 percent. The volume of soil solution in 1,000 gm. of the Norfolk soil when its moisture content was adjusted to 75 percent of its moisture equivalent was therefore 36.6 cc., while the corresponding volume of soil solution in the same weight of Cecil soil was 164.9 cc.

EXPERIMENTAL RESULTS

INFLUENCE OF DIFFERENT FERTILIZER MATERIALS ON THE CONCENTRATION OF THE SOIL SOLUTION

All fertilizer materials used directly in the tests were of c. p. grade with the exception of kainit, manure salts, cottonseed meal, and superphosphate. The materials used in the preparation of the fertilizer mixtures were of commercial grade. In calculating rates of application it was assumed that the surface 6-inch layer of an acre of dry soil weighs 2,000,000 pounds. The specific conductivities of the solutions from samples of a soil receiving the same fertilizer treatment usually agreed within 3 percent. Duplicate determinations that varied more than 5 percent were repeated.

The curves in figure 5 show the results obtained for the osmotic pressures of the solutions displaced from the Norfolk soil treated with increasing applications of various fertilizer materials. The curves show that for equal applications of plant food the phosphates and free ammonia have the least effect on the salt content of the soil solution, while sodium nitrate and the low-grade potash salts have the greatest effect. The order of these materials remained the same

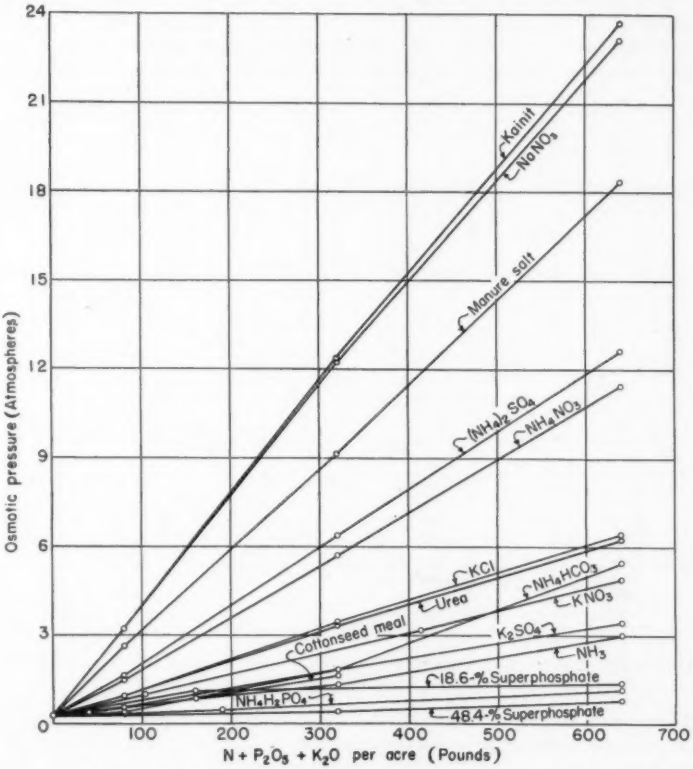

FIGURE 5.—Influence of various fertilizer materials on the osmotic pressure of the soil solution from Norfolk sandy loam soil.

when the tests were made with the Cecil soil. Long experience with several of these materials shows that they fall in the same order under field conditions, indicating that the laboratory method gives a true measure of the relative influence of fertilizers on the concentration of the soil solution.

The relative effects of increasing applications of different nitrogenous materials on the concentration of the soil solution in Norfolk soil are indicated in figure 6. The curves in this figure show that the materials fall in the same order for all applications up to the maximum

used of 640 pounds of nitrogen per acre.[4] This order remained substantially the same, as shown in figure 7, when the materials were applied to the Cecil soil. A comparison of the curves in the two figures shows, however, that when the moisture in each soil was adjusted to three-fourths of its moisture equivalent the same application of fertilizer increased the concentration of the soil solution in the Norfolk soil to a much greater extent than in the Cecil soil.

The effect of urea on the concentration of the soil solution in soils stored at 5° C. was usually between that of ammonium nitrate and ammonium bicarbonate (figs. 5 and 6) for equal applications of nitrogen. At summer temperatures urea rapidly decomposes in the soil

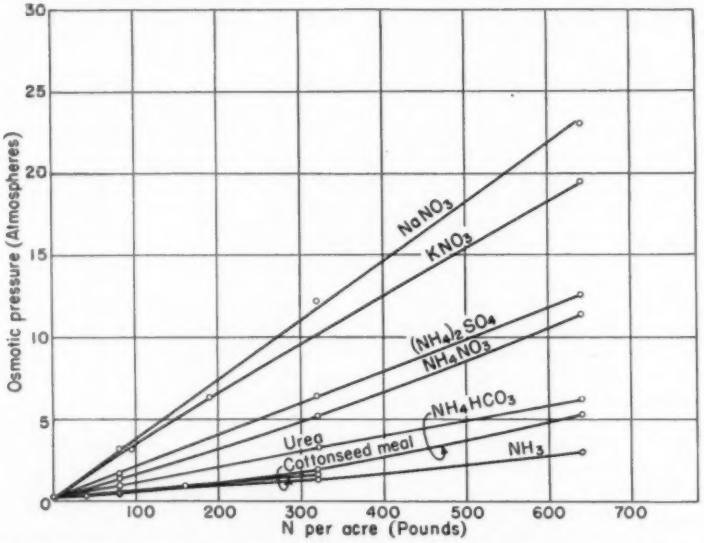

FIGURE 6.—Influence of various nitrogenous materials on the osmotic pressure of the soil solution from Norfolk sandy loam soil.

to form ammonia and ammonium bicarbonate (*12*). This decomposition of one mole of urea to form one mole each of two decomposition products would normally tend to increase the concentration of the soil solution, but owing to the relatively high fixing power of soils for ammonia the effect of urea on the concentration of the soil solution was actually found to be less at 25° than at 5°.

The striking difference in the effects of different potash salts on the concentration of the soil solution is shown in figures 8 and 9. The curves show, for example, that 80 pounds of potash as high-grade kainit (20 percent K_2O) has about the same effect on the concentration of the soil solution as 640 pounds of potash as potassium sulphate. The kainit used in the tests contained about 60 percent of sodium

[4] An application of 640 pounds of nitrogen per acre, or a comparable amount of P_2O_5 or K_2O, is much greater than that ordinarily applied under field conditions. Applications up to this maximum were considered advisable in this investigation in order to reproduce the concentrations of salts found in the seed zone when fertilizers are applied in bands at the side of the seed or plant as now recommended for most row crops (*15*).

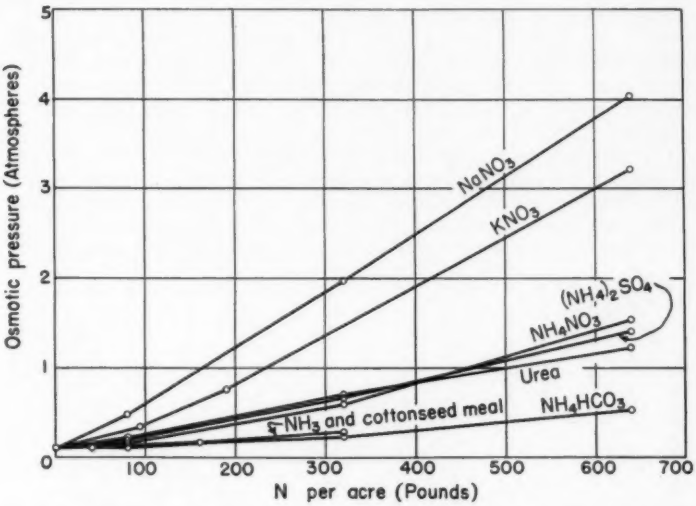

FIGURE 7.—Influence of various nitrogenous materials on the osmotic pressure of the soil solution from Cecil clay loam soil.

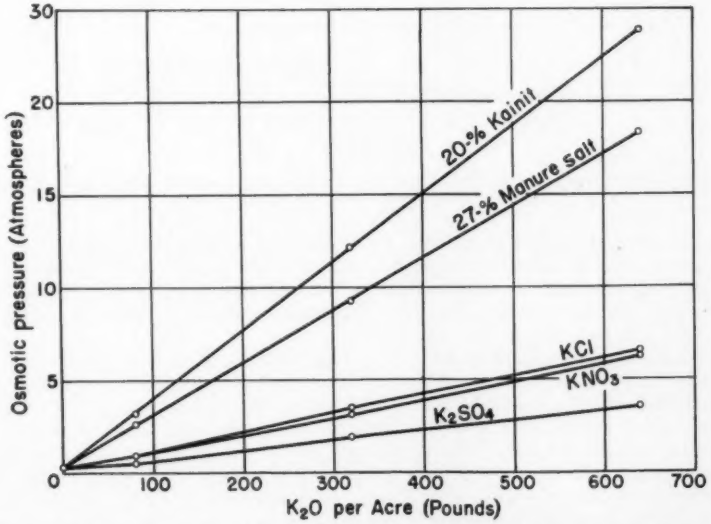

FIGURE 8.—Influence of various potash salts on the osmotic pressure of the soil solution from Norfolk sandy loam soil.

chloride in addition to potassium chloride and smaller amounts of other salts. As the grade of the kainit decreases the proportion of sodium chloride increases. Sodium chloride contains none of the primary fertilizer elements, but it produces plant burning as readily as high-grade potassium chloride containing upward of 60 percent of plant food. If low-grade rather than high-grade kainit had been used in the tests, the differences cited would have been still more striking. The potash salts fall in the same order for the Norfolk and and Cecil soils when arranged according to their effect on the soil solution. This arrangement in ascending order is potassium sulphate, potassium nitrate, potassium chloride, manure salts, and kainit.

That superphosphate and monoammonium phosphate have a relatively small effect on the salt content of soil solutions even for appli-

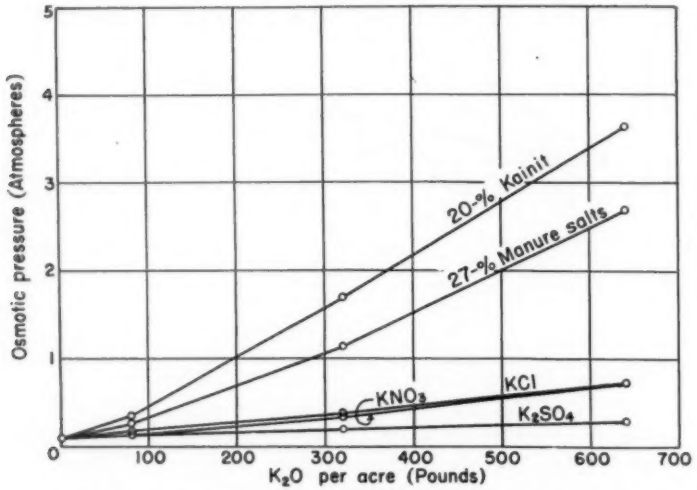

FIGURE 9.—Influence of various potash salts on the osmotic pressure of the soil solution from Cecil clay loam soil.

cations up to 1,280 pounds of P_2O_5 per acre is shown by the curves in figure 10. A direct comparison of superphosphate with sodium nitrate shows that an application to Norfolk soil of 6,000 pounds of the former has no greater effect on the concentration of the soil solution than 250 pounds of the latter.

According to Parker (*10*), the concentration of a soil solution is inversely proportional to the moisture content of the soil, and while the product of the freezing-point lowering of the soil solution and of the moisture content of a soil differs for different soils and for varying applications of a fertilizer on the same soil, it is fairly constant within limits for any given soil treatment. The value of this constant, K, may therefore be calculated from the equation $K=\Delta M$, where Δ is the freezing-point lowering of the solution displaced from the soil and M is the moisture content of the soil. Knowing the value of K as determined for a soil of a given moisture content, the approximate

value for the osmotic pressure of the soil solution in the soil at a different moisture content may then be readily calculated by substituting K/M for Δ in equation 1.

TABLE 2.—*Osmotic pressures of the soil solutions from fertilized and unfertilized Norfolk and Cecil soils of the same moisture content*

Fertilizer treatment		Osmotic pressure of—		
		Norfolk soil solution		Cecil soil solution, found for moisture =19.7 percent
Material	Plant food per acre	Found for moisture =3.8 percent	Calculated for moisture =19.7 percent	
	Pounds			
	N	*Atmospheres*	*Atmospheres*	*Atmospheres*
None	None	0.25	0.05	0.11
Ammonium sulphate	80	1.63	.31	.20
Do	320	6.35	1.23	.66
Do	640	12.60	2.44	1.39
Sodium nitrate	80	3.22	.63	.48
Do	320	12.26	2.38	1.95
Do	640	23.20	4.48	4.10
Ammonium nitrate	80	1.47	.28	.19
Do	320	5.64	1.09	.60
Do	640	11.44	2.21	1.52
Ammonia	80	.54	.11	.12
Do	320	1.33	.25	.28
	K_2O			
Potassium chloride	80	.93	.18	.17
Do	320	3.44	.66	.37
Do	640	6.54	1.27	.76
Potassium sulphate	80	.51	.09	.15
Do	320	1.82	.35	.21
Do	640	3.47	.66	.30
Potassium nitrate	80	.93	.18	.13
Do	320	3.18	.62	.35
Do	640	6.27	1.21	.75
Kainit, 20 percent K_2O	80	3.21	.62	.35
Do	320	12.36	2.39	1.71
Do	640	23.80	4.60	3.86
	P_2O_5			
Monoammonium phosphate	160	.45	.08	.16
Do	640	1.32	.25	.29
Do	1,280	3.83	.74	.45
Superphosphate	160	1.10	.22	.19
Do	640	1.38	.27	.64
Do	1,280	1.98	.39	1.09
Double superphosphate	160	.42	.08	.14
Do	640	.78	.16	.27
Do	1,280	1.74	.34	.37

In table 2 are given the osmotic pressures found for the soil solutions displaced from Norfolk and Cecil soils with moisture contents of 3.8 and 19.7 percent, respectively, or three-fourths of their water equivalents, and the calculated osmotic pressures of the corresponding soil solutions from the Norfolk soil on the basis of a moisture content of 19.7 percent, or the same as that of the Cecil soil.

The results given in table 2 show that the differences in the osmotic pressure of the soil solution of Norfolk and of Cecil soils are relatively small when their moisture content is the same. It would seem, therefore, that the difference in the effect of any given fertilizer on the concentration of the soil solution in these two soils when the moisture

content of each is adjusted to three-fourths of its water equivalent is due mainly to the difference in their moisture content rather than to any inherent difference in their chemical and physical properties. The results further show that while a given application of fertilizer increases the concentration of the soil solution in Norfolk soil to a much greater extent than in Cecil soil (figs. 6 to 10) when the moisture in each soil is at the optimum for crop growth, the reverse may be true if the Cecil soil is dry and the Norfolk soil is relatively wet.

A comparison of the last two columns of table 2 shows that the osmotic pressure of the soil solution in the Norfolk soil at a moisture content of 19.7 percent is usually greater than that of the corresponding solution from the Cecil soil of the same moisture content, and that these differences increase with increase in the fertilizer application.

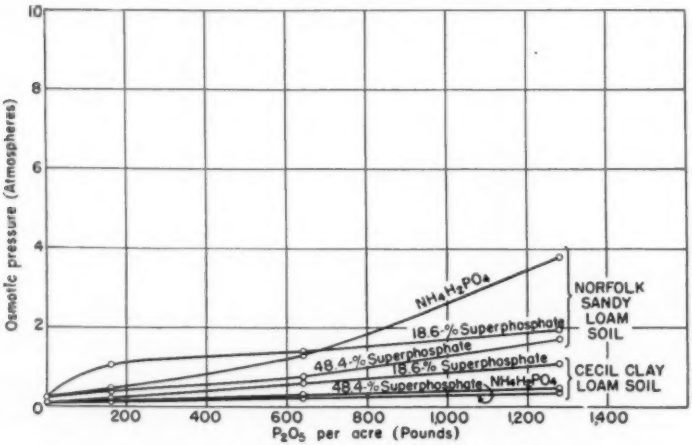

FIGURE 10.—Influence of various phosphatic materials on the osmotic pressure of the soil solution from two soils.

Although these differences are relatively small, as already pointed out, they appear to be larger than the limit of experimental error and indicate a greater fixation of fertilizer in the Cecil than in the Norfolk soil of the same moisture content.

INFLUENCE OF MIXED FERTILIZERS ON THE CONCENTRATION OF THE SOIL SOLUTION

The fertilizer formulas given below are typical of mixtures in use at different periods in the history of the fertilizer industry (*13*).

Year 1880; Grade, 2–9–2

Material:	*Pounds per ton*
Fish scrap, 6.0 percent N; 8.0 percent P_2O_5	600
Sodium nitrate, 15.5 percent N	27
Superphosphate, 12.5 percent P_2O_5	1,053
Kainit,[1] 12.5 percent K_2O	320
Total	2,000

[1] Approximate percentage composition: KCl, 20; NaCl, 75; sulphates, 1; insoluble matter, 4.

Year 1910; Grade, 3–9–3

Material—Continued.	
Ammonium sulphate, 20.0 percent N	100
Sodium nitrate, 15.5 percent N	130
Cottonseed meal, 7.0 percent N	285
Superphosphate, 16.0 percent P_2O_5	1,125
Manure salts,[2] 20.0 percent K_2O	300
Filler	60
Total	2,000

Year 1937; Grade, 4–9–5

Ammonium sulphate, 20.5 percent N	166
Ammonia, 82.3 percent N	21
Sodium nitrate, 16.0 percent N	63
Tankage, 7.0 percent N	170
Urea, 46.6 percent N	18
Superphosphate, 19.0 percent P_2O_5	947
Potassium chloride,[3] 50.0 percent K_2O	200
Dolomite	224
Filler	191
Total	2,000

[2] Approximate percentage composition: KCl, 32; NaCl, 61; sulphates, 1; insoluble matter, 6.
[3] Approximate percentage composition: KCl, 79; NaCl, 4; insoluble matter, 17.

The first of the three formulas given shows that the average mixture consumed in 1880 contained about 2 percent of N, 9 percent of P_2O_5, and 2 percent of K_2O, or a total of 13 percent of plant food. Organic nitrogen was the cheapest form of nitrogen at that time, and the organic ammoniates were therefore the principal nitrogenous materials used in fertilizer mixtures. The spread in the cost of the different forms of nitrogen soon disappeared, however, and for a period centering around 1890 the cost of nitrogen remained about the same for the different carriers in which it appeared on the market. Consequently, the practice was developed of using equal quantities of all three forms of nitrogen in the preparation of mixed fertilizers. This practice was continued through 1910, although by that time the cost of organic nitrogen had become greater than that of either of the other two forms.

The third formula, above, shows that the typical present-day mixture differs from that in use in either 1880 or 1910 in that it contains a fourth form of nitrogen (amide nitrogen) as Cyanamid or urea. Ammonia nitrogen is now the cheapest form of nitrogen, and its use in mixed fertilizers, as free ammonia, ammonium nitrate, and ammonium sulphate, exceeds by more than 50 percent that of all other forms of nitrogen combined. Dolomite is also used in mixed fertilizers to correct their acid influence on the soil. It will be noted that during the history of the industry the P_2O_5 content of the superphosphate used in mixed fertilizers has increased from 12.5 to 19.0 percent, the K_2O content of the potash salts from 12.5 to 50.0 percent, and the total plant-food content of fertilizer mixtures from 13.0 to 18.0 percent. These formulas show that a marked change has taken place in the grade as well as in the composition of fertilizer mixtures.

The extent to which these changes in the grade and composition of fertilizers has influenced their effect on the concentration of the soil solution is shown in figure 11. The curves show that the mixtures of 1880 and 1910 are much alike in their effect on the salt content of the

soil solution in both Norfolk and Cecil soils, whereas the influence of the present-day mixture is considerably less than that of either of the other two. This indicates that the danger from salt injury to plants by fertilizers is decreasing rather than increasing, as might be supposed from the increased use of the synthetic products in mixed fertilizers. The principal factors that contribute to the decreased salt effect of the average present-day mixtures are (1) the increased plant-food content of the mixture whereby less fertilizer has to be distributed per acre for a given application of plant food, (2) the replacement of kainit and other low-grade potash salts with high-grade

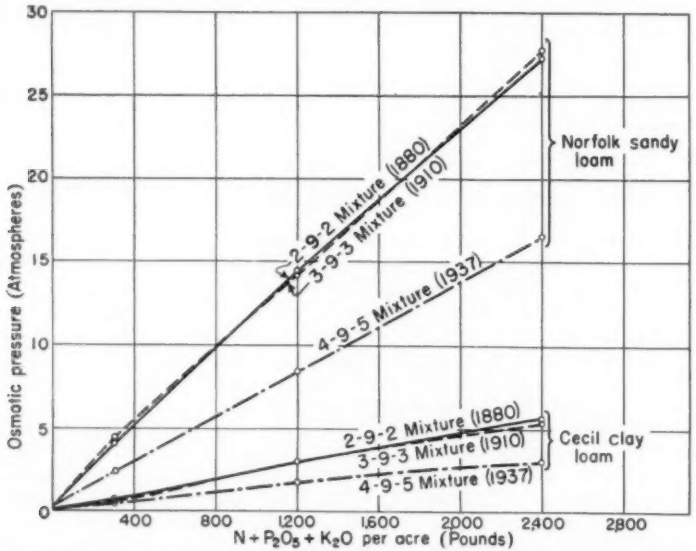

FIGURE 11.—Relative influence of typical present-day mixed fertilizers on the osmotic pressure of the soil solution in two soils.

muriate, and (3) the substitution in part of free ammonia for other soluble nitrogenous materials. These changes, as indicated by the curves in figures 6, 7, 8, and 9, have a marked effect in decreasing the influence of fertilizers on the salt content of the soil solution.

Formulas of three other fertilizer mixtures that have been or are now in general use are given in table 3. A fertilizer mixture prepared according to the 3–8–4 formula given in the table contains 400 pounds of manure salts per ton and is typical of mixtures in use about 15 years ago. In fertilizing cotton it was common practice to apply a mixture of this kind to the soil a week or so in advance of planting and then a like quantity of nitrogen later as a side dressing. If both fertilizer treatments had been made at the same time, the application would have been equivalent to that of a 6–8–4 fertilizer. The materials used in the 6–8–4 mixture (table 3) are representative of those in use at present. The materials composing the double-strength 6–16–8

mixture are also typical of those now in use. The results obtained with these three mixtures are shown in figure 12. The solid-line curves represent the changes in the specific conductivity, in reciprocal ohms, of the soil solution in the Norfolk and Cecil soils with increased applications of fertilizer, and the broken-line curves represent the changes in the osmotic pressure of the solutions. The two sets of curves show a considerable spread with increased application of the fertilizer, particularly on the Norfolk soil. This is due, as already

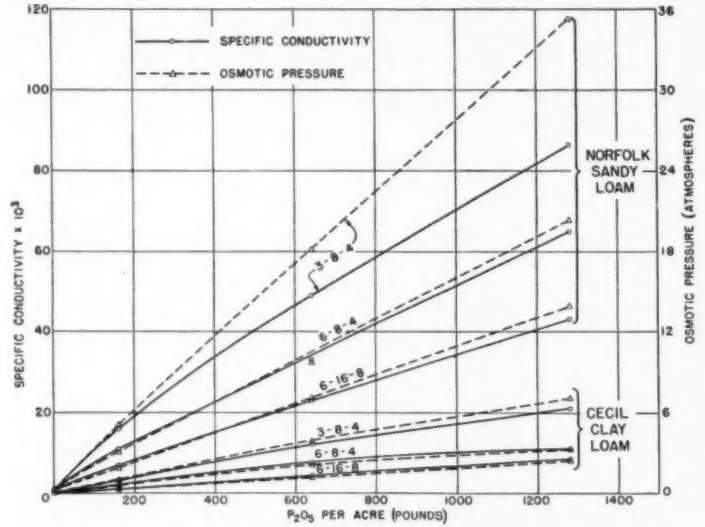

FIGURE 12.—Influence of mixed fertilizers of different types on the concentration of the soil solution in two soils.

explained, to the solvent action of high concentrations of certain salts on the organic matter of the soil.

TABLE 3.—*Formulas of fertilizer mixtures of different types*

Material	Quantity per ton in mixed fertilizer—		
	3-8-4	6-8-4	6-16-8
	Pounds	*Pounds*	*Pounds*
Ammonium sulfate, 20.8 percent N	96	252	105
Ammonium nitrate, 35.0 percent N		70	119
Ammonia, 82.3 percent N		23	40
Sodium nitrate, 16.3 percent N	123		
Cottonseed meal, 7.0 percent N	286	343	343
Superphosphate, 16.0 percent P_2O_5	1,000		
Superphosphate, 20.6 percent P_2O_5		777	294
Double superphosphate, 48.8 percent P_2O_5			535
Manure salts, 20.0 percent K_2O	400		
Potassium chloride, 60.1 percent K_2O		133	266
Dolomite		402	298
Filler (sand)	95		
Total	2,000	2,000	2,000

The curves of figure 12 also show that the double-strength 6-16-8 mixture has less effect on both the conductivity and osmotic pressure of the soil solution than the 6–8–4 mixture and that the effect of this mixture is less than that of the single-strength 3–8–4 mixture in use about 15 years ago. This relationship holds true for both soils and for all applications up to the maximum of 1,280 pounds of P_2O_5 per acre, equivalent to 8 tons of the 6–8–4 mixture or 4 tons of the 6–16–8 mixture. Such high applications are never made broadcast, but in band placements concentrations of this order may often be approached in the vicinity of the seed zone.

It should be emphasized that while a double-strength mixture prepared according to the formula of table 3 has less effect on the

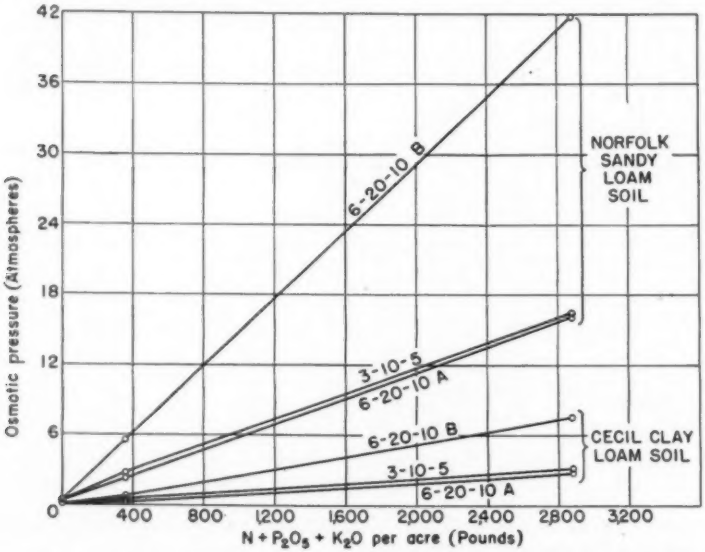

FIGURE 13.—Relative influence of single- and double-strength mixed fertilizers on the osmotic pressure of the soil solution in two different soils.

concentration of the soil solution than the corresponding single-strength mixture, this need not necessarily hold for all types of mixtures. Thus the curves in figure 13 show that the double-strength 6–20–10 mixture prepared according to formula A of table 4 has less effect on the concentration of the soil solution of the soils used in the tests than the corresponding single-strength 3–10–5 mixture, but the reverse is true when the double-strength mixture consists of Ammophos, sodium nitrate, and low-grade potash salts, as specified in formula B of table 4. Ammophos, sodium nitrate, and the different grades of potash salts are extensively used in fertilizer mixtures, but a mixture prepared from these materials only does not contain an adequate proportion of the secondary plant-food elements, and it is therefore not to be recommended for continued use on soils deficient in these elements.

TABLE 4.—*Formulas of single- and double-strength fertilizer mixtures*

Material	Quantity per ton in mixed fertilizer—		
	3-10-5	6-20-10	
		A	B
	Pounds	*Pounds*	*Pounds*
Ammonium sulfate, 20.8 percent N	100	200	
Ammonia, 82.3 percent N	30	60	
Ammophos A, 10.7 percent N; 48.0 percent P_2O_5			833
Sodium nitrate, 16.3 percent N	49	98	190
Cottonseed meal, 7.0 percent N	100	200	
Superphosphate, 20.4 percent P_2O_5	981		
Double superphosphate, 46.7 percent P_2O_5		858	
Potassium chloride, 60.2 percent K_2O	166	332	
Manure salts, 20.5 percent K_2O			977
Dolomite	126	252	
Filler (sand)	448		
Total	2,000	2,000	2,000

It should be further emphasized that the relationship usually found between the total plant-food content of typical fertilizer mixtures and their effect on the soil solution does not necessarily hold true if the ratios of the plant-food elements in the mixtures are not the same or if the plant-food content is increased without corresponding decrease in the rate of application to the soil. Thus, the danger from burning for a given application of a 4–8–8 fertilizer mixture would be greater than for the same application of a 4–8–4 fertilizer of similar composition, but this would not hold true for half the application of the corresponding double-strength or 8–16–8 mixture.

It may therefore be concluded (1) that the effect of fertilizers, prepared according to present commercial practice, on the salt content of the soil solution decreases as a rule with increase in the plant-food content of the mixture when the ratio of plant food remains the same, and (2) that danger of salt injury in the use of typical present-day mixtures is less than that from mixtures formerly used, even when the nitrogen in the older type mixtures is applied to the crop in split applications.

SUMMARY

A study was made of the effect of fertilizers on the concentration of the soil solution in Norfolk sandy loam and Cecil clay loam soils. The procedure followed consisted of mixing the fertilizer to be tested with a sample of soil, adjusting the moisture content of the soil to 75 percent of its moisture equivalent, allowing it to stand at 5° C. for 5 days, separating the soil solution from the soil by the displacement method, determining the concentration of the solution by freezing-point depression measurements, and comparing its concentration with that of the solution recovered from the unfertilized soil.

For equal applications of plant food the phosphates and free ammonia had the least effect on the concentration of the soil solution of both the soils used in the tests, while sodium nitrate and the low-grade potash salts had the greatest effect.

The extent to which a fertilizer increased the concentration of the soil solution was much greater with the Norfolk soil than with the

163005—39——2

Cecil soil. The difference in the osmotic pressure of the soil solutions of the two soils largely disappeared, however, when their moisture content was the same.

Comparison of the formulas of fertilizer mixtures representative of those in use at different periods in the history of the fertilizer industry shows that a marked change has taken place not only in the grade but also in the composition of mixed fertilizers.

Mixtures typical of those in use in 1880 and in 1910 are much alike in their effect on the concentration of the soil solution, whereas the influence of the average present-day mixture is considerably less than that of either of the other two mixtures. The reduced effect of present-day mixtures on the salt content of the soil solution is due (1) to the increased plant-food content of the mixture whereby less fertilizer has to be distributed per acre for a given application of plant food, (2) to the replacement of kainit and other low-grade potash salts with high-grade muriate, and (3) to the substitution in part of free ammonia for other soluble nitrogenous materials.

While the effect of fertilizers on the concentration of the soil solution does not necessarily decrease with increase in the grade of the mixture, this relationship holds true, as a rule, for mixtures of the same plant-food ratio when these are prepared as in present commercial practice. It is possible to prepare a 6–8–4 mixture from present-day materials that has less effect on the salt content of the soil solution than the 3–8–4 mixtures formerly used. The results indicate that danger of salt injury from typical present-day mixtures is less than that from mixtures formerly used, even when the nitrogen in the latter mixtures is applied to the crop in split applications.

LITERATURE CITED

(1) Briggs, Lyman J., and McLane, J. W.
1911. moisture equivalent determinations and their application. Amer. Soc. Agron. Proc. (1910) 2: 138–147, illus.

(2) ——— and McLane, J. W.
1907. the moisture equivalents of soils. U. S. Dept. Agr., Bur. Soils Bull. 45, 23 pp., illus.

(3) Burd, John S., and Martin, J. C.
1923. water displacement of soils and the soil solution. Jour. Agr. Sci. [England] 13: [265]–295, illus.

(4) Conrad, John P., Proebsting, E. L. and McKinnon, L. R.
1930. equipment and procedure for obtaining the displaced soil solution. Soil Sci. 29: 323–329, illus.

(5) Harris, J. Arthur, and Gortner, Ross Aiken.
1914. notes on the calculation of the osmotic pressure of expressed vegetable saps from the depression of the freezing point, with a table for the values of P for $\Delta=0.001°$ to $\Delta=2.999°$. Amer. Jour. Bot. 1: 75–78.

(6) Ishcherekov, V.
1907. the obtaining of the soil solution in an unaltered condition. Zhur. Opytn. Agron. Iugo-Vostoka. (Jour. Expt. Landw. Südost, Eur.-Russlands) 8: 147–166. [Abstract in Expt. Sta. Rec. 19: 409. 1907.]

(7) Lewis, Gilbert Newton.
1908. the osmotic pressure of concentrated solutions, and the laws of perfect solution. Jour. Amer. Chem. Soc. 30: 668–683.

(8) Lipman, Chas. B.
1918. a new method of extracting the soil solution. Calif. Univ. Pubs., Agr. Sci. 3: 131–134.

(9) MORGAN, J. FRANKLIN.
1917. THE SOIL SOLUTION OBTAINED BY THE OIL PRESSURE METHOD. Soil Sci. 3: 531–545.
(10) PARKER, F. W.
1921. METHODS OF STUDYING THE CONCENTRATION AND COMPOSITION OF THE SOIL SOLUTION. Soil Sci. 12: 209–232.
(11) PARKER, HENRY C., and PARKER, ELIZABETH W.
1924. THE CALIBRATION OF CELLS FOR CONDUCTANCE MEASUREMENTS. III. ABSOLUTE MEASUREMENTS ON THE SPECIFIC CONDUCTANCE OF CERTAIN POTASSIUM CHLORIDE SOLUTIONS. Jour. Amer. Chem. Soc. 46: 312–335, illus.
(12) REHLING, C. J., and TAYLOR, J. R., JR.
1937. FORMAMIDE AS A NITROGENOUS FERTILIZER. Jour. Amer. Soc. Agron. 29: 134–144, illus.
(13) ROSS, WILLIAM H., and MEHRING, ARNON L.
1938. MIXED FERTILIZERS. U. S. Dept. Agr. Yearbook 1938 (Soils and Men): 522–545, illus.
(14) SCHLŒSING, TH.
1866. SUR L'ANALYSE DES PRINCIPES SOLUBLES DE LA TERRE VÉGÉTALE. Compt. Rend. Acad. Sci. [Paris] 63: 1007–1012.
(15) WHITE, L. M., and ROSS, W. H.
1936. INFLUENCE OF FERTILIZERS ON THE CONCENTRATION OF THE SOIL SOLUTION. Soil Sci. Soc. Amer. Proc. 1: 181–186, illus.

A THREE-DIMENSIONAL LATTICE DESIGN FOR STUDIES IN FOREST GENETICS[1]

By BESSE B. DAY, *associate statistician*, and LLOYD AUSTIN, *in charge, Institute of Forest Genetics, Forest Service, United States Department of Agriculture*[2]

INTRODUCTION

The logical procedure for the improvement of all wild stock of any kind, whether plant or animal, is practically the same: segregation of varieties, races, and strains of the wild population; the evaluation of the characteristics of each group; the selection of the best individuals from each of the best strains; utilization of these in breeding and selection; and finally the production of new types (*5*).[3]

In forest genetics, both in breeding and in mass reforestation, the initial step involves seed selection. It is evident that there are many valuable inherent characteristics in forest trees. These need to be discovered, isolated, and defined. The measure of heredity is to be found in the offspring. Seeds from individual seed trees must be collected and sown and the behavior of the progeny therefrom studied. This necessitates the making of fairly extensive progeny tests.

The same difficulty arises in individual seed-tree progeny studies as in other plant research in varietal testing—the lack of homogeneity of the medium, soil, in which such tests are to be made. It is a well-established fact that there is much variation even in soil which seemingly has the most constant texture and quality and that the variation is reflected in the growth of the plants to such an extent that variety difference may be so completely obscured as to be lost entirely. It is only when the area is exceedingly small that soil effects may be ignored. As the number of varieties to be tested increases, the area necessary for a complete set becomes increasingly larger and the variation of soil and other growing factors are likewise magnified, resulting in what may be a considerable lack of precision. All attemps to solve this problem proved unsatisfactory for one reason or another until Yates (*7, 8, 10*) conceived the idea of arranging the varieties in a series of small blocks, instead of the previous arrangement in one block, distributing them in such a way that a variety variance could be calculated which would be freed of block effects. This would yield an error variance appropriate for making significance tests of differences found in the measurements of the varieties of progenies. Designs on this principle, termed "quasi-factorial" and "incomplete randomized blocks," are readily adaptable to all phases of agronomy where varietal tests are made.

The theoretical aspects of this type of design have been treated in earlier writings (*3, 7, 10*). It is believed, however, that the application of this design to a particular field problem will be of interest in

[1] Received for publication December 5, 1938.
[2] Grateful acknowledgment is due Prof. R. A. Fisher, Galton Laboratory, University College, London, for proposing this type of design for the experiment; to F. Yates, chief statistician, Rothamsted Experimental Station, for invaluable counsel and interest in the development of this experiment; and to Prof. P. M. Barr, Division of Forestry, University of California, who made numerous suggestions leading to a more precise presentation.
[3] Italic numbers in parentheses refer to Literature Cited, p. 118.

that it affords an exact test of the effectiveness of the design in ironing out heterogeneity. For this purpose the description is here given of such a design as applied to the progeny test nursery planted in April 1937 at the Institute of Forest Genetics, Placerville, Calif.,[4] and the procedure that was followed for correcting the resulting data and making tests of significance of differences. Actual data are employed and the appropriate statistical procedure has been carried through on germination counts. In these data factors known to produce variability of conditions were actually introduced, as in the watering of sections of the experiment at unequal intervals. Their influence was reflected in the results. With the application of the correction procedure such effects were eliminated.

The objectives of the 1937 tests were as follows: (1) To determine the hereditary nature of the numerous local strains of ponderosa pine occurring in the various localities through El Dorado County, Calif., including probable casual relationships with elevation and other factors of the seed-trees' environment; (2) to discover which individual seed trees in each strain, or in each elevational belt, have the inherent ability to produce the most rapidly growing offspring, as determined by measurement of the height, diameter, and branching of the progenies.

The experiment was limited to 729 seed selections or strains, 696 ponderosa pine (*Pinus ponderosa*) and 33 Jeffrey pine (*P. jeffreyi*), gathered from 17 consecutive 500-foot altitudinal zones in what was known as the El Dorado transect, an area in El Dorado County, extending less than 100 miles across the main range of the Sierra Nevada and about 50 miles parallel to the range. Some of the seeds were obtained from the same trees but in different years. In many cases two or more of the seed trees were growing in the same field plot. It was desired to design a nursery in such a way that measurements on progenies would be available free from the influence of all heterogeneity in growing conditions and with an estimate of error adequate for testing the significance of differences between particular progenies.

THEORETICAL BASIS AND DESCRIPTION OF THE DESIGN

The design best adapted to the testing of an unusually large number of varieties is termed the "three-dimensional quasi-factorial" with three groups of sets ("pseudo-factorial" in earlier publications), or more simply, the cubic lattice design (6). The initial requirement is that the number of varieties tested be a perfect cube. In this experiment 729, or 9^3, individual seed selections were made. Nine plots or varieties were grouped together to make up a block. It follows, then, that 81 blocks are needed for one complete replication. It was believed that this block size was sufficiently small to eliminate unavoidable heterogeneity therein. Fundamentally this process of arranging fewer than the total number of treatments (individual seed selections) in a block, in other words more than one block to a replication, results in confounding (*10*). Some of the information on treatment or interaction effects is sacrificed by being entangled with fertility differences between blocks in order that the precision may be increased through a reduction of the standard error due to elimination of block

[4] A part of the California forest and Range Experiment Station.

differences. Where the heterogeneity of the medium of the experiment is pronounced, the net gain will more than balance the loss in efficiency, as will be demonstrated in this experiment. Here there will be a partial confounding of main effects with block effects.

To overcome this complexity, all the plots in a replication were grouped in the 81 blocks in 3 different ways. The chief requirements for these three were that each individual seed selection be planted not more than once in the same block with any other particular seed selection and that the blocks of each group should cut across those of the other groups. This will be demonstrated in greater detail a little later. Comparisons between the nine progencies within a block may be made directly, but the information on the difference between progenies not occurring in the same block will be entangled with block differences and must be arrived at indirectly through cross-block comparisons. Likewise, the direct information for progenies in the same block will be enhanced by similar cross-block sources. The information available on the differences between pairs of progenies will not be equally precise for all possible comparisons. Instead there will be 3 degrees of precision, hence 3 standard errors, corresponding to the relative position of the seedlings.

The 3 groups, which in the subsequent discussion will be called the X, Y, and Z groups, were each replicated 3 times, making altogether 9 replications, or a total of 6,561 plots in 729 blocks. The only difference between the 3 replications of a group was the independent randomizing of the plots within each block. This design, as will be shown, yields a proper correction factor for each progency which, when applied to its average, eliminates differences due to block effects.

So extensive an experiment requires some systematic numbering scheme. The method adapted from Yates (*7*) was to designate each variety (seed selection) by a three-digit number as uvw, in which u, v, and w always remain in the order uvw but each may take 9 different values. There will be $9^3=729$ different combinations of the 9 numbers, or sufficient for the total number of strains selected. This system is illustrated in figure 1, where a cube of dimension 9 is divided into 729 sections and numbered accordingly. Beginning at the upper front edge and reading to the right, u takes on the values 1 to 9 while v and w remain constant, as 111, 211, 311 ------ 911; reading down from the same starting point w varies from 1 to 9 while u and v are constant, as 111, 112, 113------ 119; finally reading in the third direction, as the v arrow points, u and w are constant and v changes from 1 to 9, as 111, 121, 131------ 191. Each individual seed selection was given one of these 729 numbers which it carried throughout the experiment. No restrictions were placed on the order in which they were made. However, it was desired for technical reasons to have the plots in one replication appear as nearly as possible, without destroying the validity of the test, in the order of the elevation of the seed source, and so the numbers were assigned after the groupings, described in the next paragraph, were effected, and before the plots within the blocks had been randomized.

Referring again to figure 1, the divisions of plots into sets may be readily accomplished for the three groupings mentioned above, heeding only the restrictions that no two strains appear together in the same block in more than one of the groups, and that the sets of each

division be so arranged that they cut across those of all the other divisions. Cutting the cube by two sets of parallel planes through the intersections—the first set parallel to the right-front face and the second parallel to the upper face—yields 81 blocks, each nine plots (small cubes) long. The numbers 111, 211, 311------ 911, form a set, another is 112, 212, 312------ 912, and so until 81 such blocks are designated. It will be noted that in each of these sets or blocks

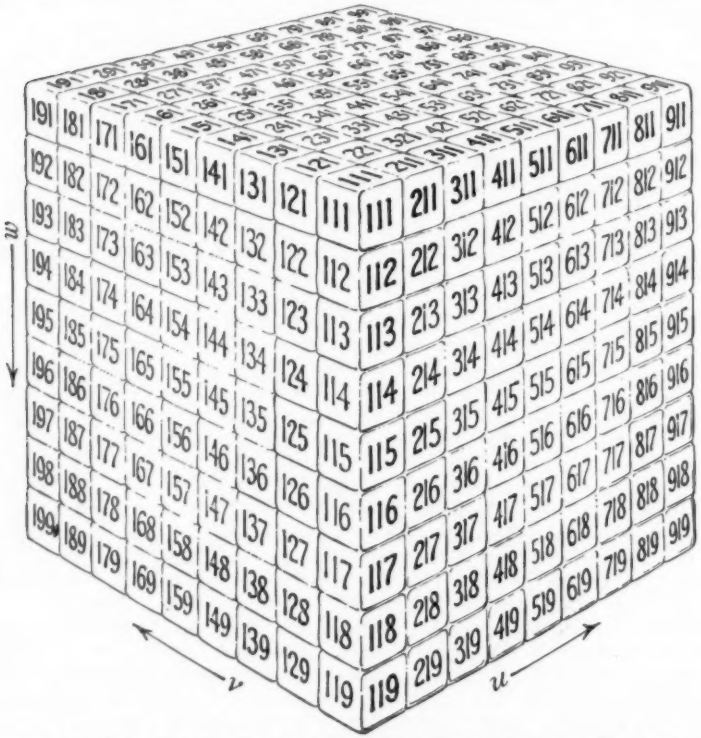

FIGURE 1.—Cube to illustrate the method for determining the sets in the *X*, *Y*, and *Z* groups for the three-dimensional lattice progeny test nursery.

vw numbers are constant and *u* varies from 1 to 9. This group of 81 sets or blocks was designated by the letter *X*, and each block number is formed by the combination of its constant *vw* and small *x*, e. g., 11*x*, 12*x*, etc. Group *X* is given in detail in the first vertical section of table 1.

Passing planes through the cube parallel to the left front face and then horizontally cuts across the sets in the first group making certain that no two plots which appeared together in the *X* group are now together. The new sets form the *Y* group. In these *u* and *w* are constant and *v* varies from 1 to 9. One such block is 111, 121, 131 ------ 191; another 911, 921, 931 ------ 991. Consistent with

the first group these would be blocks 11*y* and 91*y*. The second vertical section of table 1 shows the sets of the *Y* group.

TABLE 1.—*Numbering system for progenies in the 1937 progeny test nursery, showing block assignments of plots for groups X, Y, and Z*

Block No.	Group *X* (·*xw*)					Block No.	Group *Y* (*u*·*w*)					Block No.	Group *Z* (*ux*·)				
11*x*	111	211	311	---	911	11*y*	111	121	131	---	191	11*z*	111	112	113	---	119
12*x*	112	212	312	---	912	12*y*	112	122	132	---	192	12*z*	121	122	123	---	129
13*x*	113	213	313	---	913	13*y*	113	123	133	---	193	13*z*	131	132	133	---	139
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
10*x*	119	219	319	---	919	10*y*	119	129	139	---	199	10*z*	191	192	193	---	199
21*x*	121	221	321	---	921	21*y*	211	221	231	---	291	21*z*	211	212	213	---	219
22*x*	122	222	322	---	922	22*y*	212	222	232	---	292	22*z*	221	222	223	---	229
23*x*	123	223	323	---	923	23*y*	213	223	233	---	293	23*z*	231	232	233	---	239
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
20*x*	129	229	329	---	929	20*y*	219	229	239	---	299	20*z*	291	292	293	---	299
31*x*	131	231	331	---	931	31*y*	311	321	331	---	391	31*z*	311	312	313	---	319
32*x*	132	232	332	---	932	32*y*	312	322	332	---	392	32*z*	321	322	323	---	329
33*x*	133	233	333	---	933	33*y*	313	323	333	---	393	33*z*	331	332	333	---	339
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
30*x*	139	239	339	---	939	30*y*	319	329	339	---	399	30*z*	391	392	393	---	399
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
91*x*	191	291	391	---	991	91*y*	911	921	931	---	991	91*z*	911	912	913	---	919
92*x*	192	292	392	---	992	92*y*	912	922	932	---	992	92*z*	921	922	923	---	929
93*x*	193	293	393	---	993	93*y*	913	923	933	---	993	93*z*	931	932	933	---	939
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
·	·	·	·		·	·	·	·	·		·	·	·	·	·		·
99*x*	199	299	399		999	99*y*	919	929	939	---	999	99*z*	991	992	993	---	999

The third and last or *Z* group of sets, which cuts across each of the other two groups, is formed from the cube by passing sets of planes in both vertical directions. The blocks will stand vertically in the figure. Block 11*z* will be 111, 112, 113 ------ 119 while block 91*z* is made up of plots 911, 912, 913 ------ 919, as in the last vertical section of table 1.

Groups *X*, *Y*, and *Z* were each replicated three times in the nursery, making nine complete replications for each progeny.

DESCRIPTION OF NURSERY BEDS

The most desirable set-up, both from the standpoint of the technical phases and of the field work, was to use beds 4.5 feet by 48 feet, running north and south. Each bed was divided into 288 plots 1½ feet long and 6 inches wide running across the bed, making 3 plots to the width (north, center, and south) and 96 to the length. Nine plots at each end were kept as guard plots, leaving 270 test plots in each bed, which would yield 10 blocks of 9 plots each in each of the north, center, and south positions (figs. 2 and 3A). Each test plot held a row of 6 spots spaced 3 inches apart and planted to 6 seeds each; later these were to be thinned to one seedling per spot. Thus a replication of the 729 seed selections, each represented by 1 plot, required 2.7 seed-beds; and the entire set-up of 9 replications, 24.3 beds.

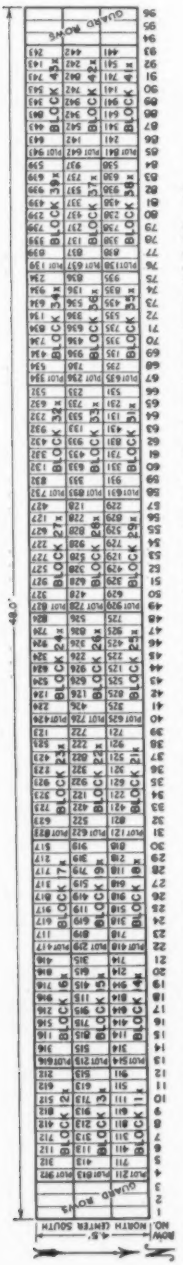

FIGURE 2.—Illustration of arrangement of plots and blocks in a typical nursery bed (bed No. 1).

For the first replication the strains were listed so that certain ones (usually in order of elevation) would appear together in the same block and particular blocks would come in consecutive order, but every precaution was taken to insure that the plot positions within these blocks, as well as those in all the other replications, be determined in a random manner. To equalize any effect due to longitudinal position in the seedbeds each particular block, of which there were three replications, appeared once in each of the three positions, north, center, and south. It was not essential that every plot in the nursery be subject to exactly the same treatment simultaneously—that is, that they be sown, watered, etc., on the same day, or receive the same amount of water—but only that precaution be taken that those within the same block be treated closely alike. To insure a completely random experiment it was decided to randomize the blocks within each of the longitudinal positions with the exception of replication *X*, in which the blocks occurred in consecutive order. Tippett's Random Sampling Numbers (*4*) was used for this work.

For recording the field data with efficiency and rapidity in a design as complicated as this, forms must be devised to fit particular measurement, the total for the plot, and the total for the block. In the example here described, the germination count data, days from time of planting until germination, were recorded on the original sowing charts of which figure 2 is a sample.

OUTLINE OF PROCEDURE FOR CORRECTING MEASUREMENTS[5]

As previously stated, the chief purposes of this design were to eliminate differences in progenies which might be due to soil or other treatment heterogeneity under which the individual progenies have been growing and to arrive at a valid estimate of error for making individual

[5] Very recently Yates has done considerable additional work on the development of methods for the recovery of the interblock information. Since this paper was presented for publication, he has very kindly made available to the authors his results (in manuscript form) on the three-dimensioned lattice. By a somewhat different computational procedure from that outlined here, it is possible to determine an estimate of the interblock variance, freed from varietal effects. Adjustments to the varietal means where the interblock and intrablock comparisons are correctly weighted may then be made. While the amount of computation required for this analysis is a little more than that described here, it yields a larger efficiency factor which is also always greater than that for the ordinary randomized blocks, except for the limiting case when there is no reduction of variance due to the use of smaller blocks. When interblock information is ignored, the efficiency factor is not always greater.

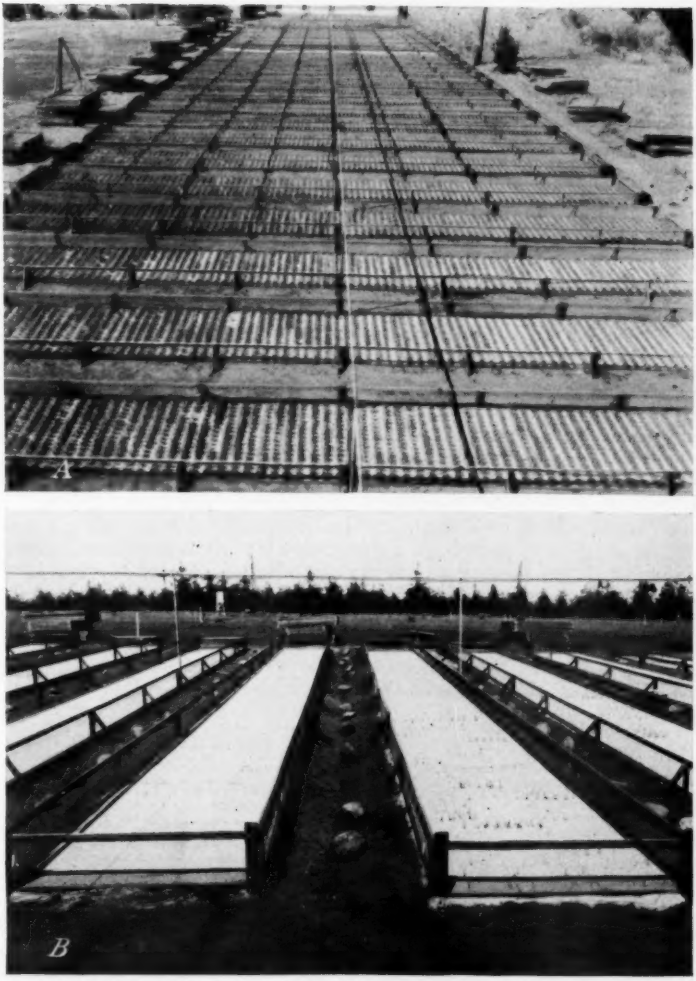

FIGURE 3.—*A*, General view of the entire progeny test experiment. *B*, Bed 16, right, was watered immediately after sowing; bed 17, left, was watered several days later—an example of an unavoidable difference that may occur in treatment and illustrating the need for a design that will eliminate variations due to such causes.

comparisons. The analysis of variance takes the following general form:

Degrees of freedom	Sum of squares	Mean square	*F*
Incomplete blocks ($3np^2-1=728$			
Progenies ($p^3-1=728$			
Error ($3np^3-3np^2-p^3+1=5,104$			
Total ($3np^3-1=6,560$			

Where $p=9$, the number of plots to the block
$p^2=81$, the number of blocks in each replication
$p^3=729$, the total number of progenies or strains
$n=3$, the number of replications of each of the three groups
$3n=r=9$, the total number of replications
$3np^2=729$, the total number of blocks in the nursery
$3np^3=N=6561$, the total number of plots in the nursery

The usual procedure may be followed in computing the sum of squares corresponding to the 728 degrees of freedom for the blocks and the 6,560 making up the total. As has been pointed out earlier, the block differences have been confounded with progenies (varieties) and hence there will be no valid mean square for blocks.

The first step in determining the sum of squares due to progeny differences is to apply a correction factor to the gross average for each variety which will eliminate the differences due to soil and treatment heterogeneity, leaving a value which is truly representative of the particular strain insofar as its characteristics were embodied in the seeds sown. A cubic lattice with dimensions p may be likened to a factorial experiment (*7, p. 432*) involving three factors each with p values. The main effects of each of these factors would be confounded in two of the three groups or replications and the p^3-1 degrees of freedom (here strains) for treatment would be as follows:

First factor (A)	$p-1$
Second factor (B)	$p-1$
Third factor (C)	$p-1$
Interactions:	
First order (AB, AC, BC)	$3(p-1)(p-1)$
Second order (ABC)	$(p-1)(p-1)(p-1)$
Total	p^3-1

The precision of the estimate of the main effects would be just one-third that of an unconfounded experiment with the same error variance per plot; that for the first order interactions would be two-thirds, while the second order would be entirely free of confounding.

In this progeny test the interest lies in the differences between single progenies rather than main effects and interactions. Estimates of the yield for each variety or strain may be expressed in terms of the gross mean yield, the main effects, and the interactions, the latter two being equivalent to removing differences due to soil or treatment heterogeneity. For factorial experiments involving two or more factors (*9, pp. 12–13*) the yield of any treatment combination is equal to the mean yield and the sum of plus or minus *one-half* of all the main

TABLE 2.—*Computation of corrected progeny means; First of series of nine; w=1*

SECTION 1: X_{uvw}

v \ u	By groups									
	1	2	3	4	5	6	7	8	9	
1	82	62	72	89	65	76	92	70	66	674 $X_{\cdot 1w}$
2	63	99	64	83	98	59	89	70	86	711
3	64	60	74	95	82	104	68	88	67	702
4	80	73	101	71	79	90	75	67	103	739
5	85	74	83	66	93	86	73	72	73	705
6	67	71	88	80	66	79	98	70	74	693
7	77	96	111	70	98	116	83	97	69	817
8	72	91	66	93	72	80	99	76	70	719
9	89	92	94	95	77	67	89	84	102	789
$X_{u \cdot w}$	679	718	753	742	730	757	766	694	710	6549=$X_{\cdot\cdot w}$

SECTION 2: Y_{vuw}

v \ u										
1	77	64	66	86	62	75	104	77	74	685 $Y_{\cdot 1w}$
2	56	89	63	80	89	64	88	65	94	688
3	51	59	69	81	74	77	63	79	73	626
4	76	78	88	66	70	81	74	72	111	716
5	77	75	81	60	92	77	66	67	75	670
6	59	65	87	76	62	82	107	72	96	706
7	67	78	91	63	88	100	73	83	66	709
8	84	99	64	90	73	83	102	74	84	755
9	67	82	80	83	63	62	86	73	90	686
$Y_{u \cdot w}$	614	689	689	685	673	703	763	662	763	6241=$Y_{\cdot\cdot w}$

SECTION 3. Z_{uvw}

v \ u										
1	79	67	72	80	66	68	86	80	63	661 $Z_{\cdot 1w}$
2	69	99	61	79	80	59	90	68	67	672
3	57	49	73	67	82	87	65	86	67	633
4	76	80	92	58	92	87	72	81	102	740
5	79	74	84	66	86	79	62	65	68	663
6	64	67	97	83	61	91	108	66	89	726
7	63	83	107	58	95	86	69	80	67	708
8	79	92	71	98	79	84	84	86	66	739
9	71	83	79	86	62	60	78	67	87	673
$Z_{u \cdot w}$	627	694	736	675	703	701	714	679	676	6215=$Z_{\cdot\cdot w}$

SECTION 4: T_{vuw}

v \ u	For all groups									
	1	2	3	4	5	6	7	8	9	
1	238	193	210	255	193	219	282	227	203	2,020 $T_{\cdot 1w}$
2	188	287	188	242	267	182	267	203	247	2,071
3	172	168	216	243	238	268	196	253	207	1,961
4	232	231	281	195	241	258	221	220	316	2,195
5	241	223	248	192	271	242	201	204	216	2,038
6	190	203	272	239	189	252	313	208	259	2,125
7	207	257	309	191	281	302	225	260	202	2,234
8	235	282	201	281	224	249	285	236	220	2,213
9	227	257	253	264	202	189	253	224	279	2,148
$T_{u \cdot w}$	1,930	2,101	2,178	2,102	2,106	2,161	2,243	2,035	2,149	19,005=$T_{\cdot\cdot w}$

SECTION 5: $T_{uw/3n}$

v \ u										
1	26.444	21.444	23.333	28.333	21.444	24.333	31.333	25.222	22.556	−0.124 $C_{\cdot 1w}$
2	20.889	31.889	20.889	26.889	29.667	20.222	29.667	22.556	27.444	−.333
3	19.111	18.667	24.000	27.000	26.444	29.778	21.778	28.111	23.000	−1.237
4	25.778	25.667	31.222	21.667	26.778	28.667	24.556	24.444	35.111	−.527
5	26.778	24.778	27.556	21.333	30.111	26.889	22.333	22.667	24.000	−.847
6	21.111	22.556	30.222	26.556	21.000	28.000	34.778	23.111	28.778	−.053
7	23.000	28.556	34.333	21.222	31.222	33.556	25.000	28.889	22.444	−1.664
8	26.111	31.333	22.333	31.222	24.889	27.667	31.667	26.222	24.444	.156
9	25.222	28.556	28.111	29.333	22.444	21.000	28.111	24.889	31.000	−1.680
$C_{u \cdot w}$	.296	−.037	.438	.043	.290	.074	−.531	.056	−1.111	

SECTION 6: CALCULATED CORRECTED PROGENY MEANS, t_{vuw}

v \ u										
1	27.440	21.760	23.734	30.771	21.982	25.094	31.020	25.555	22.521	25.542 $t_{\cdot 1w}$
2	20.577	32.324	21.606	27.797	31.484	20.904	29.058	22.915	28.644	26.145
3	19.265	19.648	23.924	27.331	25.944	29.371	20.870	27.152	21.376	23.876
4	26.531	25.883	32.109	23.566	26.518	29.569	25.037	24.868	35.166	27.694
5	27.390	24.149	26.913	21.178	30.235	27.069	22.630	22.913	23.587	25.118
6	22.073	23.783	31.700	27.355	21.961	28.085	33.795	24.589	28.505	26.872
7	23.159	26.931	32.923	20.836	30.727	32.882	23.319	29.028	19.572	26.597
8	26.998	32.892	22.463	32.483	26.109	28.424	33.689	27.372	24.880	28.361
9	25.180	28.100	27.468	28.425	21.766	19.662	26.791	24.858	29.143	25.710
$t_{u \cdot w}$	24.290	26.163	26.976	26.638	26.303	26.784	27.357	25.472	25.933	

$\Sigma(t_{vuw}\ T_{vuw})$=508, 939.949. $\Sigma(X_{\cdot 1w}\ t_{\cdot 1w})$=171, 768.205. $\Sigma(Y_{u \cdot w}\ t_{u \cdot w})$=163, 827.666.

effects and interactions. Analogous to this, the formula (7, *p. 436*) for the estimate of an individual strain, t_{uvw}, is

$$t_{uvw}=m_{uvw}+\frac{1}{2}(\overline{m}_{.vw}+\overline{m}_{u.w}+\overline{m}_{uv.})-\frac{1}{2}(\overline{m}_{u..}+\overline{m}_{.v.}+\overline{m}_{..w})$$
$$+\frac{1}{2}(\overline{X}_{u..}+\overline{Y}_{.v.}+Z_{..w})-\frac{1}{2}(\overline{X}_{.vw}+\overline{Y}_{u.w}+\overline{Z}_{uv.}) \quad (1)$$

Where m_{uvw} is the mean yield of the nine replications of a strain, the first and the third quantities within parentheses are considered main effects and the other two interactions. This formula has been developed in a detailed manner, but the procedure has not been included here since it involves considerable algebra inappropriate to the purposes of this article.

For a better understanding of the terms making up the above formula a brief explanation of the mathematical notation is needed. Subtotals of all the plots (3 of these) in each of the three groups which are numbered exactly alike are designated X_{uvw}, Y_{uvw}, and Z_{uvw}, respectively, while the total of the three groups (9 of these) is called T_{uvw}. In table 2 the first item in section 1, 82, is the sum of the three plots in the X group with the number 111; in section 2, 77 is the sum of the three plots in the Y group with the number 111, and finally in section 3, 79 is the sum of the three 111's in the Z group; and the total of all these is 238 in section 4. A dot appearing in place of the u, v, or w in a subscript indicates a summation of all plots whose numbers in the same position as the dot range from 1 to 9; i. e., $X_{.vw}$ is the summation of all plots with the same vw but with u ranging from 1 to 9, and $X_{..w}$ that of all plots with w constant but all values of u and v. Thus 674 is the sum of the 27 progeny measurements in group X whose numbers end in 11; and 6,549 is the sum of the 243 progeny values in group X with w=1, u and v taking on all values from 1 to 9. This notation is extended for all terms with uvw subscripts and is quite adequate for all summations. The corrected progeny mean is denoted t_{uvw} while $t_{.vw}$, $t_{u.w}$, and $t_{uv.}$ are the average progeny means for the nine progenies having constant vw, uw, and uv designations. For ease of computation it has been found desirable to combine certain of the correction terms into three symmetrical parts, designated $C_{.uw}$, $C_{u.w}$, and $C_{uv.}$ Their composition and consistency will appear from the discussion to follow.

It is now possible to show the application of formula (1) to the data at hand, as follows:

$m_{uvw}=\frac{T_{uvw}}{3n}$ (section 5 of the first nine computational tables of which table 2 is a sample);

$\overline{m}_{.vw}$, $\overline{m}_{u.w}$, and $\overline{m}_{uv.}$ = the same average of $3np$ (or 81) plots making up $T_{.vw}$, $T_{u.w}$, and $T_{uv.}$, respectively (section 4 of the series of 10 tables);

$\overline{m}_{u..}$, $\overline{m}_{.v.}$, and $\overline{m}_{..w}$ = the average of $3np^2$ (or 729) plots in $T_{u..}$, $T_{.v.}$, and $T_{..w}$, respectively (section 4 of the series of 10 tables);

$\overline{X}_{u..}$, $\overline{Y}_{.v.}$, and $\overline{Z}_{..w}$ = the average of np^2 (or 243) plots making up $X_{u..}$, $Y_{.v.}$, and $Z_{..w}$, respectively (sections 1 and 2 of the last table of series [table 3], and section 3 of the first 9 [table 2];

TABLE 3.—*Computation of corrected progeny means: Summary for all values of w*

SECTION 1: $X_{w.}$

w \ u	By groups									
	1	2	3	4	5	6	7	8	9	
1	727	672	719	785	662	712	724	752	712	6,465 $X_{1.}$
2	719	784	761	666	773	728	768	706	792	6,697
3	816	776	674	662	723	824	758	726	743	6,702
4	801	822	875	710	780	826	781	751	808	7,154
5	800	832	789	807	849	743	863	733	782	7,098
6	776	807	839	763	784	799	725	805	796	7,094
7	817	752	766	748	782	841	749	757	800	7,012
8	759	766	718	817	790	788	793	793	764	6,988
9	759	795	748	758	693	766	824	730	804	6,877
$X_{.u}$	6,974	7,006	6,889	6,716	6,836	7,027	6,885	6,753	7,001	62,087 = $X_{..}$

SECTION 2: $Y_{w.}$

w \ u	By groups									
	1	2	3	4	5	6	7	8	9	
1	671	660	676	750	671	712	715	697	706	6,258 $Y_{1.}$
2	737	753	715	675	735	759	745	677	762	6,558
3	752	702	650	602	706	733	687	686	668	6,186
4	745	725	748	679	699	740	708	714	726	6,484
5	737	806	724	715	780	684	712	697	746	6,601
6	705	795	753	703	714	778	702	760	776	6,686
7	737	697	747	679	749	790	744	740	738	6,621
8	730	708	692	762	722	792	747	741	735	6,629
9	723	734	714	679	657	716	748	660	759	6,390
$Y_{.u}$	6,537	6,580	6,419	6,244	6,433	6,704	6,508	6,372	6,616	58,413 = $Y_{..}$

SECTION 3: $Z_{w.}$

w \ u	By groups									
	1	2	3	4	5	6	7	8	9	
1	680	677	726	626	678	684	737	740	666	6,214 $Z_{1.}$
2	798	753	724	636	645	705	781	688	617	6,347
3	743	606	639	571	720	755	698	736	701	6,169
4	741	760	768	564	783	711	665	708	684	6,384
5	722	848	811	771	801	674	647	679	693	6,646
6	730	744	743	730	732	821	791	711	768	6,770
7	701	768	807	676	736	779	750	655	831	6,703
8	757	670	783	766	735	785	621	739	691	6,547
9	680	712	718	722	659	757	759	614	760	6,381
$Z_{.u}$	6,552	6,538	6,719	6,062	6,489	6,671	6,449	6,270	6,411	58,161 = $Z_{..}$

$\Sigma(Z_{w.}t_{w.}) = 1{,}587{,}292.030$.

SECTION 4: $T_{w.}$

w \ u	For all groups									
	1	2	3	4	5	6	7	8	9	
1	2,078	2,009	2,121	2,161	2,011	2,108	2,176	2,189	2,084	18,937 $T_{1.}$
2	2,254	2,290	2,200	1,977	2,153	2,192	2,294	2,071	2,171	19,602
3	2,311	2,084	1,963	1,835	2,149	2,312	2,143	2,148	2,112	19,057
4	2,287	2,307	2,391	1,953	2,262	2,277	2,154	2,173	2,218	20,022
5	2,259	2,486	2,324	2,293	2,430	2,101	2,122	2,109	2,221	20,345
6	2,211	2,346	2,335	2,196	2,230	2,398	2,218	2,276	2,340	20,550
7	2,255	2,217	2,320	2,103	2,267	2,410	2,243	2,152	2,369	20,336
8	2,246	2,144	2,193	2,345	2,247	2,365	2,161	2,273	2,190	20,164
9	2,162	2,241	2,180	2,159	2,009	2,239	2,331	2,004	2,323	19,648
$T_{.u}$	20,063	20,124	20,027	119,022	19,758	20,402	19,842	19,395	20,028	= 178,661 $T_{..}$

SECTION 5: CALCULATION OF CORRECTION FACTOR, $C_{w.}$

w \ u	For all groups									
	1	2	3	4	5	6	7	8	9	
1	.824	.477	.087	2.519	.372	.811	.342	.401	1.200	
2	−.275	.805	.612	1.198	1.860	.941	.255	.636	2.644	
3	1.095	2.258	.723	1.525	.447	.756	.860	.222	.724	
4	.984	.780	.976	2.383	−.023	1.355	1.539	.895	1.693	
5	1.163	.255	−.234	.649	.681	.953	1.675	1.037	1.545	
6	.719	1.317	1.093	.809	.724	.064	−.399	1.475	.891	
7	1.527	.076	−.184	1.235	.879	.916	.514	1.747	−.097	
8	.435	1.440	−.524	1.062	.774	.527	2.397	.938	1.391	
9	1.342	1.261	.599	.729	.712	.268	.891	1.593	.934	

SECTION 6: CALCULATED CORRECTED PROGENY MEANS, $t_{w.}$

w \ u	For all groups									
	1	2	3	4	5	6	7	8	9	
1	26.044	24.799	26.056	28.653	24.770	26.290	26.680	26.873	26.308	
2	27.258	28.736	27.697	25.200	28.151	27.597	28.190	25.791	28.965	
3	28.556	26.867	24.105	22.998	25.918	28.118	26.155	25.552	25.541	
4	27.832	27.828	29.326	24.996	26.521	27.968	26.653	26.216	27.502	
5	28.021	29.868	27.644	27.815	29.655	25.749	26.750	25.924	27.747	
6	27.168	29.386	29.281	26.962	27.412	28.710	26.044	28.607	28.746	
7	28.554	26.587	27.863	26.274	28.059	29.745	27.301	27.384	28.151	
8	27.416	27.115	26.021	29.155	27.773	28.866	28.237	28.134	27.494	
9	27.023	27.870	26.720	26.262	24.509	26.788	28.566	25.205	28.416	

And, finally,

$\overline{X}_{\cdot vw}$, $\overline{Y}_{u\cdot w}$, and $\overline{Z}_{uv\cdot}$ = the average of np (or 27) plots in $X_{\cdot vw}$, $Y_{u\cdot w}$, and $Z_{uv\cdot}$, respectively, sections 1 and 2 of the first 9 tables [table 2] and, section 3 of the last of series [table 3].

For computational work a slightly different form has been found most suitable. Substituting the foregoing in formula (1) gives—

$$t_{uvw}=\frac{T_{uvw}}{3n}+\frac{1}{2}\left[\frac{T_{\cdot vw}}{3np}+\frac{T_{uv\cdot}}{3np}+\frac{T_{u\cdot w}}{3np}\right]-\frac{1}{2}\left[\frac{T_{u\cdot\cdot}}{3np^2}+\frac{T_{\cdot v\cdot}}{3np^2}+\frac{T_{\cdot\cdot w}}{3np^2}\right]$$
$$+\frac{1}{2}\left[\frac{X_{u\cdot\cdot}}{np^2}+\frac{Y_{\cdot v\cdot}}{np^2}+\frac{Z_{\cdot\cdot w}}{np^2}\right]-\frac{1}{2}\left[\frac{X_{\cdot vw}}{np}+\frac{Y_{u\cdot w}}{np}+\frac{Z_{uv\cdot}}{np}\right]$$

which may be reduced to

$$t_{uvw}=\frac{T_{uvw}}{3n}+\frac{1}{6np^2}\left[pT_{\cdot vw}-3pX_{\cdot vw}-T_{\cdot v\cdot}+3Y_{\cdot v\cdot}\right]+\frac{1}{6np^2}(pT_{u\cdot w}$$
$$-3pY_{u\cdot w}-T_{\cdot\cdot w}-3Z_{\cdot\cdot w}\Big]+\frac{1}{6np^2}[pT_{uv\cdot}-3pZ_{uv\cdot}-T_{u\cdot\cdot}+3X_{u\cdot\cdot}]$$

With the terms containing the brackets represented by $C_{\cdot vw}$, $C_{u\cdot w}$, and $C_{uv\cdot}$ the formula for correcting an individual progeny becomes—

$$t_{uvw}=\frac{T_{uvw}}{3n}+C_{\cdot vw}+C_{u\cdot w}+C_{uv\cdot} \qquad (2)$$

It will be seen later that the computation of the C's is a very simple matter. $C_{v\cdot w}$ and $C_{u\cdot w}$ appear in the margins of section 5, table 2, $C_{uv\cdot}$ in section 5, table 3. Table 2, section 6, gives the corrected mean germination days of the 81 progenies so numbered that $w=1$. There will be similar sections for w equal to 2, 3, ------ 9.

Using the corrected progeny means it would be possible to find the sum of squares for progenies by the usual procedure. It can be proved, however, that the same results may be arrived at with the formula—

SS (corrected progeny total)

$$=\Sigma(t_{uvw}T_{uvw})-[\Sigma(X_{\cdot vw}t_{\cdot vw})+\Sigma(Y_{u\cdot w}t_{u\cdot w})+\Sigma(Z_{uv\cdot}t_{uv\cdot})] \qquad (3)$$

and with very much less work.

Had there been no confounding in this experiment, the variance of every comparison between pairs of progencies would have been $\frac{2s^2}{r}$, s being the error value determined from the analysis of variance table and r the number of replications. Because of the confounding, pairs of progenies will be classified in three ways on the basis of their relative block locations, for comparison by means of the variance of the mean difference. The variance for each of these is expressed in the following three formulas—

$$V(t_{211}-t_{111})=\frac{2s^2}{rp^2}(p^2+p+1) \qquad (4)$$

$$V(t_{122}-t_{111})=\frac{s^2}{rp^2}(2p^2+3p+4) \qquad (5)$$

$$V(t_{222}-t_{111})=\frac{s^2}{rp^2}(2p^2+3p+6) \qquad (6)$$

depending on whether the progenies differ in one, two, or three of the letters *uvw*.

The mean variance of all comparisons is—

$$V_m=\frac{s^2}{r}\frac{(2p^2+5p+11)}{p^2+p+1} \quad \text{----------(7)}$$

Formulas 4, 5, and 6 for variances of mean difference were derived in the same manner as were the formulas for correcting progeny means, in terms of main effect and interactions (*7, pp. 433–437*).

The resulting standard errors from extracting the square roots of each of the four variances are the error factors to be used in making individual comparisons of progenies by "Student's" t test.

The factor by which each of the above variances differ from $\frac{2s^2}{r}$

is a measure of the increase in variance that results from the division of the varieties into sets when the error variance per plot is unaltered by the resultant reduction in block size. The reciprocal of such factor, called efficiency factor of the arrangement (*9, p. 86*), is a measure of the inherent strength of the arrangement.

Thus the increases in variance are—

$$\frac{2s^2}{rp^2}(p^2+p+1)\div\frac{2s^2}{r}=\frac{p^2+p+1}{p^2}$$

$$\frac{s^2}{rp^2}(2p^2+3p+4)\div\frac{2s^2}{r}=\frac{2p^2+3p+4}{2p^2}$$

$$\frac{s^2}{rp^2}(2p^2+3p+6)\div\frac{2s^2}{r}=\frac{2p^2+3p+6}{2p^2}$$

and for all comparisons—

$$\frac{s^2}{r}\frac{2p^2+5p+11}{p^2+p+1}\div\frac{2s^2}{r}=\frac{2p^2+5p+11}{2(p^2+p+1)}$$

With $p=9$, the efficiency factor is in each case then—

$$\frac{p^2}{p^2+p+1}=.890,$$

$$\frac{2p^2}{2p^2+3p+4}=.839,$$

$$\frac{2p^2}{2p^2+3p+6}=.831,$$

and for the mean variance of all comparisons—

$$\frac{2(p^2+p+1)}{2p^2+5p+11}=.835$$

163005—39——3

APPLICATION OF PROCEDURE TO GERMINATION DATA

With the preceding notation and formulas as a basis, the actual computations involved in correcting the progeny means for heterogeneity are very simple. The process can best be carried through in tabular form using a series of 10 computational tables of six sections each, of which table 2 illustrates the first ($w=1$) and table 3 the last—a summary of the other nine tables. For illustrative material, the time of germination was recorded for each of the 6,561 plots. This time was defined as the number of days from planting until the first day when three or more spots (six spots to the plot) had one or more seedlings (six seeds planted to the spot) visible above the ground regardless of whether or not they had been injured by damping-off or otherwise.

The procedure may be demonstrated as follows:

(1) Using a convenient index for finding the location in the nursery of the replications of individual progenies, the first step is to find the sum of the three plot measurements having the same *uvw* number in each of the three groups and record them in tabular form. For example, in table 2, for totals in the X group, the readings for No. 111 were $31+27+24=82$; in the Y group, the readings for the same progeny were $28+27+22=77$; and in the Z group they were $28+26+25=79$. This process is repeated for each of the other 728 progenies.

(2) The sum of the three totals above, $82+77+79=238$, or T_{111}, the total for the 9 replications of progeny No. 111. This and similar totals for the progenies whose numbers end in 1 are recorded in the fourth section of table 2.

(3) Marginal totals are found for the first four sections yielding $X_{u\cdot w}$, $X_{\cdot v w}$, $X_{\cdot\cdot w}$, and these are repeated for Y, Z, and T, as indicated in table 2.

(4) The average $\frac{T_{uvw}}{9}$ is computed for each value of T in the nine tables—a very simple matter. These form the fifth section of each. In table 3, for No. 111, $\frac{238}{9}=26.444$.

(5) For $X_{uv\cdot}$ the items occupying the same position in each of the nine tables are added. The first summation is $82+93+84+70+68+74+97+90+69=727$. Of these, the first value only, 82, may be found in table 2. This result, 727, is the first item in the tenth table, here represented by table 3. This process is repeated for each of the other progenies.

(6) Marginal totals here yield $X_{u\cdot\cdot}$ and $X_{\cdot v\cdot}$. The same procedure is followed for Y, Z, and T.

(7) The next important step is the calculation of the correction factors $C_{\cdot vw}$, $C_{u\cdot w}$, and $C_{uv\cdot}$. Substituting in the formulas given above for these:

$$C_{\cdot vw}=\frac{1}{6\times 3\times 9^2}[9\times 2020\ \text{(table 2)}-27\times 674\ \text{(table 2)}-18{,}937\ \text{(table 3)}+3\times 6258\ \text{(table 3)}]=-0.124\ \text{(vertical margin of fifth section, table 2).}$$

$C_{u'w}=\frac{1}{6\times 3\times 9^2}[9\times 1930$ (table 2)-27×614 (table 2)$-19{,}005$ (table 2)$+3\times 6215$ (table 2)$]=0.296$ (horizontal margin of fifth section, table 2).

$C_{uv'}=\frac{1}{6\times 3\times 9^2}[9\times 2078$ (table 3)-27×680 (table 3)$-20{,}063$ (table 3)$+3\times 6974$ (table 2)$]=0.824$ (fifth section, table 3).

Attention is called to the fact that the coefficients of these three formulas are such as to give equal weighting to all items. The sum of all the C's should equal zero, which is a check on the accuracy of the computations.

(8) The final operation in correcting the individual progenies for heterogeneity is to apply these correction values to the original average in section 5, table 2, using formula 1—

$$26.444-0.124+0.296+0.824=27.440$$

which is the corrected number of days from planting until germination for progeny No. 111.

With each of the 729 progeny means corrected, it is a very simple matter to carry through the computations necessary for obtaining the items in the analysis of variance table form above. If d_{111}, d_{211} ------ d_{999} be the original germination time, then summing over all replications—

$\Sigma d_{uvw}=178{,}661$, and the correction factor becomes $\frac{(178{,}661)^2}{6561}=4{,}865{,}074.37$.

$\Sigma d^2_{uvw}=5{,}122{,}921$.

Total $SS=5{,}122{,}921-4{,}865{,}074.37=257{,}846.63$.

For the variation due to blocks, b,

$\Sigma b^2=44{,}601{,}697$

SS due to blocks $=\frac{44{,}601{,}697}{9}-4{,}865{,}074.37=90{,}669.74$

The usual procedure for finding the sums of squares for the corrected progeny measurements could have been used, but formula (3) simplifies and shortens the labor to a considerable degree. Substituting therein—

SS due to progenies $=5{,}011{,}580.822-[1{,}689{,}976.220+1{,}592{,}598.640+1{,}587{,}292.030]=141{,}713.932$.

These known items, may now be tabulated for the analysis of variance and the error term, 25,462.96, obtained by subtraction, table 4.

TABLE 4.—*Analysis of variance of the germination period in days*

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Blocks	728	90,669.74		
Progenies	728	141,713.93	194.66	[1] 39.02
Error	5,104	25,462.96	4.988824	
Total	6,560	257,846.63		

[1] Highly significant.

The error mean square in table 4 is the squared standard error, that is, $s^2=4.988824$. The comparison of individual progeny means falls into three groups, which may be determined from the progeny number, with distinct standard errors of the difference. These are expressed in formulas (4) to (7). Substituting in these

$$V(t_{211}-t_{111})=\frac{2\times 4.988824}{729}\times 91=1.245495 SE=1.116$$

$$V(t_{122}-t_{111})=\frac{4.988824}{729}\times 193=1.320772 SE=1.149$$

$$V(t_{222}-t_{111})=\frac{4.988824}{729}\times 195=1.334459 SE=1.155$$

Mean variance of all comparisons

$$V_m=\frac{4.988824}{9}\times\frac{218}{91}=1.327917 \qquad SE_m=1.152$$

With these standard errors it is now possible to make any individual comparisons of means desired using the well-known t test.

$t=\frac{\text{Mean}_1-\text{Mean}_2}{SE}$ and referring to the t table (*1, p. 166*) for the probability that the difference might be due to random sampling. This t is, of course, the one first established by "Student" in 1908. An adequate treatment of it is given by Fisher (*1*). To compare progenies No. 111 and No. 211.

$$t=\frac{21.760-27.440}{1.116}=-5.090$$

Entering the t table (*1, p. 166*) at $n=16$ ($n=n_1+n_2$ where n_1+1 and n_2+1 are each equal to 9), the computed value of t, 5.090, is found to be far beyond the range of the table, showing that the propability, P, is extremely small. This justifies the conclusion that the seeds from the tree whose progeny is No. 111 germinate at a slower rate than those from the seed tree of progeny No. 211.

In an earlier paragraph the efficiency factors for this design were computed. For the mean variance of all comparisons, this efficiency factor was found to be 0.835. In other words, 16.5 percent was lost because the ordinary randomized block per replication was not used. The reduction in error variance due to the design will, however, more than compensate for this loss. It is possible to take into account the information accruing from the block comparisons, since this experiment has a sufficient number of replications (nine in all with three for each of the groups X, Y, and Z) to give an adequate estimate of error for interblock as well as the intrablock comparisons. This adds greatly to the attractiveness of the design.

To use the information from the interblock comparisons most accurately, all the blocks forming a complete replication X, Y, or Z should be arranged in a compact unit on the ground, with these three groups randomized for positions with reference to each other in addition to the randomization of blocks within groups and plots within blocks (*9, pp. 30, 31, 86*). Although utilization of interblock compari-

sons has been introduced since the experiment was installed and the pattern outlined was not exactly followed, still it is possible to recover much of the lost information. With the present design, the 728 degrees of freedom ascribed to blocks (these confounded with main effects) may be broken down as follows:

	D/f
Groups	2
X grouping:	
Blocks	80
Error	162
Y grouping:	
Blocks	80
Error	162
Z grouping:	
Blocks	80
Error	162
	728

Combining the three terms for error gives 486 degrees of freedom. $\frac{\Sigma(X_{.uv})+\Sigma(Y_{u.v})+\Sigma(Z_{uv.})}{27}$ minus the correction factor will give the part of the sum of squares for blocks other than error. Subtracting this from the total sum of squares due to blocks leaves the error term attributed to the 486 degrees of freedom.

This computation becomes

$$\frac{48{,}003{,}111+42{,}409{,}381+42{,}040{,}325}{27}-4{,}865{,}074.37=40{,}585.52.$$

90,669.74 (*SS* due to blocks)$-40{,}585.52=50{,}084.22.$

This may be set up in the following form:

Item	*D/f*	*SS*	*MS*
Blocks	486	50,084.22	103.05
Within	6,074		4.988824
Total	6,560		12.25

The mean square for the 6,560 degrees of freedom, 12.25, is found thus:

$$\frac{50{,}084.22+(6074\times4.988824)}{6560}=12.25$$

Following Fisher (*2, pp. 255–258*), the expression for precision is $\frac{n+1}{(n+3)s^2}$, where n is the degrees of freedom and s^2 the sampling variance. Hence the ratio of the two sampling variances above, $\frac{12.25}{4.99}=2.45$, or 245 percent, will measure the recovery of information due to reduction in error variance by the use of this design. This means that the experiment is about 2½ times as precise as it would have been if the ordinary randomized block design had been used.

Earlier, account was taken of the loss of information due to the confounding of main effects with blocks, with the result that the

efficiency of the experiment was assessed at 0.835. The net efficiency from these two sources is then

$$0.835 \times 2.45 = 2.05$$

This net gain of 105 percent makes it evident that the use of the cubic lattice design for this experiment was most worth while.

EXPLANATION AND SUMMARY

An examination of the body of germination data after the correction factors have been applied gives most conclusive evidence of the effectiveness of this type of design for the purpose for which it was evolved, namely, to eliminate differences in yields or measurements due to soil or treatment heterogeneity. Besides the expected soil variations, a known variable factor was introduced in the watering time of the nursery. It is granted that watering will tend to hasten germination. The entire nursery was watered once, beds 1–16 on April 27 and beds 17–25 on May 7. The sowing started on April 20 with bed 1 and continued consecutively until finished on May 5. This means that the greatest number of days between planting and watering for the first set would have been for bed 1, and the least for bed 16; in the second set the greatest number of days for bed 17, the lowest number of days for bed 25 (fig. 3 *B*).

The number of days from watering to germination were also recorded for each of the 6,561 plots. Using the original average values, time to germinate from planting date minus the time to germinate from watering varied from plot to plot, a range of 3.3 to 5.9.

When the average number of days from watering time to germination for each progeny was corrected, as was done for the planting time, the differences between corrected planting and corrected watering time became practically constant at 4.6 days. This is evidence that this design does eliminate effects of plot differences upon the average values, whether they be initial or some later happening, as in the incident of watering the nursery.

The correction of the 729 progeny means for heterogeneity due to location and the setting up of the procedure for making tests of significance of the differences of these individual progeny means completes the objectives of this article. From the standpoint of the purposes for which this experiment was designed, however, it marks only the initial step in the selection of seed trees for the improvement of the strains of timber trees; it is necessary to learn what conditions affect the growing characteristic of the seedlings. Hence, the next step, which is beyond the scope of this article, would be to subject the corrected data to such standard statistical procedures as seem most applicable.

LITERATURE CITED

(1) FISHER, R. A.
1936. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 6, rev. and enl., 339 pp. illus. London and Edinburgh.

(2) ———
1937. THE DESIGN OF EXPERIMENTS. Ed. 2, 260 pp., illus. London and Edinburgh.

(3) GOULDEN, C. H.
1937. MODERN METHODS FOR TESTING A LARGE NUMBER OF VARIETIES. Canada Dept. Agr. Pub. 575 (Tech. Bull. 9), 36 pp., illus.

(4) TIPPETT, L. H. C.
1927. RANDOM SAMPLING NUMBERS. London Univ., Dept. Appl. Statis. Tracts for Computers No. 15, 8 pp., 26 tables. London.
(5) UNITED STATES DEPARTMENT OF AGRICULTURE.
1936–37. YEARBOOK OF AGRICULTURE, 1936 AND 1937.
(6) YATES, F.
1935. COMPLEX EXPERIMENTS. Jour. Roy. Statis. Soc. Sup. 2: 181–247.
(7) ———
1936. A NEW METHOD OF ARRANGING VARIETY TRIALS INVOLVING A LARGE NUMBER OF VARIETIES. Jour. Agr. Sci. [England] 26: 424–455, illus.
(8) ———
1936. INCOMPLETE RANDOMIZED BLOCKS. Ann. Eugenics 7: 121–140.
(9) ———
1937. THE DESIGN AND ANALYSIS OF FACTORIAL EXPERIMENTS. Imp. Bur. Soil Sci. Tech. Commun. No. 35, 96 pp. Harpenden, England.
(10) ———
1937. A FURTHER NOTE ON THE ARRANGEMENT OF VARIETY TRIALS: QUASI-LATIN SQUARES. Ann. Eugenics 7: 319–332, illus.

ELECTRICAL STIMULATION OF ISOLATED HEART PREPARATIONS FROM PERIPLANETA AMERICANA[1]

By J. Franklin Yeager

Senior entomologist, Division of Control Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture

INTRODUCTION

In order to study adequately the effects of insecticides upon the various tissues and functional processes of insects, particularly insects which it is desired to control, it is necessary to have more knowledge of the manner in which the tissues and organs of insects function under normal and abnormal conditions. This paper is a report of experiments which yielded further information regarding the functional processes of the insect-cardiac mechanism.

In utilizing the mechanocardiographic method of studying the effects of nicotine and other solutions upon a perfused, isolated heart preparation from the American cockroach (*Periplaneta americana* (L.)) it is necessary to consider the effects of possible contractions by other than cardiac-muscle fibers. In addition to the cardiac-muscle fibers, the cockroach isolated heart preparation previously used (*4*, *5*, *6*, *7*)[2] possessed alary muscles (of the heart), dorsal body muscles, and, in the thoracic region, especially of heart preparations from the adult insect, portions of the muscles involved in the somatic wing mechanisms.

It has been shown previously (*4*) that the systolic rise of curve in the mechanocardiogram taken from the whole isolated heart preparation of *Periplaneta americana* is often immediately preceded by a sudden depression, the presystolic notch. It has been suggested (*4*), on the basis of certain unpublished evidence, that the presystolic notch might result from an increased intracardiac pressure (hydrostatic) produced by a propagated wave of heartbeat in a cardiac region other than that from which the mechanocardiographic record is being taken. Although under certain conditions the presystolic notch may originate in this manner, the possibility still remains that under other conditions it might originate from a rhythmic presystolic contraction of the alary-muscle fibers of the heart, especially in the segment from which the record is being made. If the presystolic notch originates in accordance with this hypothesis, it should be possible to obtain supportive evidence by artificially stimulating the isolated heart preparation, especially the alary-muscle fibers in the segment in which the heart lever is attached.

The experiments herein reported were performed with this in mind, but also with the general objective of determining how the isolated heart preparation of *Periplaneta americana* responds to single and repeated faradic stimuli and how these responses compare with the better known results of artificial stimulation of the vertebrate heart.

[1] Received for publication December 12, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 137.

METHODS

As described below, four different types of isolated heart preparations were used in these experiments.

Type A, the entire isolated heart preparation that was utilized in previous investigations (*5, 6, 7*) was used in some of these experiments. The preparation consisted of the beheaded insect's dorsal body wall (terga), dissected from the rest of the body but retaining the cardiac mechanism. It contained the cardiac tube, the alary-muscle-fiber groups, the external and internal dorsal body muscles, the dorsal diaphragm, the fat bodies, trachea, and other incidental structures associated with the dorsal diaphragm and the terga, and, in the thorax, the severed somatic wing muscles.

Type B, the second preparation, consisted of only the abdominal region of the heart, prepared either by transverse section across preparation A between the third thoracic and the first abdominal segments or by separating by similar cross section the abdomen from the thorax of the living insect and subsequently isolating the abdominal heart preparation by dissection. Whichever procedure was followed, the resulting preparation was the same and contained the entire abdominal but not the thoracic portions of the cardiac mechanism. In this preparation the longitudinal internal and external dorsal body muscles of the abdomen remained intact and retained their normal origins and insertions.

Type C, the third preparation, consisted of a single abdominal segment separated by transverse section with a safety-razor blade from adjacent posterior and anterior segments of either preparation A or preparation B (usually the latter) but cut in such a way that the adjacent margin of the adjacent overlapping segment remained attached to the segment being isolated. This preparation of a single abdominal segment contained a single pair of alary muscle-fiber groups and only that length of cardiac tube that lay between their cardiac terminations. The internal dorsal body muscles were transectioned, and only the ends attached to the isolated segment remained in the preparation. The external dorsal body muscles were not severed.

Type D, the fourth preparation, was made in the same way as preparation C except that the adjacent margin of the adjacent overlapping segment was dissected away and the connections of the external dorsal body muscles therefore destroyed. This preparation was thus completely separated from both anterior and posterior adjacent segments.

During some of these experiments the isolated heart preparation was kept moist by occasional flooding with approximately 0.11 Lévy's (*3*) stock saline solution (prepared without the buffers). This saline was composed of 11.78 gm. of sodium chloride, 0.92 gm. of potassium chloride, and 0.66 gm. of calcium chloride per liter of solution. In the other experiments the heart preparation was continuously perfused with a stream of another saline solution that was made to flow over the exposed tissues. The formula for the composition of this saline solution was arrived at in the following way: Mixtures were made containing known proportions of the 0.11 Lévy's solution described above and of a saline (composed of 3.26 gm. of sodium chloride, 7.39 gm. of potassium chloride, 2.54 gm. of calcium chloride, and 1.69 gm. of magnesium chloride per liter of solution)

prepared by Babers on the basis of his analysis (*1*) of the blood of the mature larva of the southern armyworm (*Prodenia eridania* (Cram.)). These mixtures were used to perfuse the isolated heart preparation of *Periplaneta americana*, and that mixture was chosen which sufficed to maintain the perfused heart preparation in the most steady state with respect to both rate and amplitude of cardiac contractions. The selected mixture was found by calculation to have the composition 10.93 gm. of sodium chloride, 1.57 gm. of potassium chloride, 0.85 gm. of calcium chloride, and 0.17 gm. of magnesium chloride per liter of solution. This solution will be referred to in this paper as the magnesium saline, the 0.11 Lévy's solution will be referred to as Lévy's saline. The perfusion saline was oxygenated by bubbling oxygen through the reservoir bottle before and during the experiment.

The isolated heart preparation was held by pins in a beeswax container so constructed that the perfusion saline flowed over the exposed cardiac mechanism into a surrounding drainage depression from which it flowed into a collecting jar. The drainage depression of the wax container surrounded a central wax elevation or plateau one edge of which was higher than the other. Drainage was facilitated by laying narrow strips of wet lens paper in the drainage depression. The heart preparation used in each experiment was pinned to the wax plateau with the external dorsal surface down and the internal cardiac mechanism (dorsal diaphragm) up. The rate of flow of perfusion fluid over the exposed cardiac tissues was adjusted by means of a stopcock until it was as rapid as possible without mechanically interfering with the hair attachment or the mechanocardiogram. This method of perfusion was somewhat different from that used in previous studies of the rate of insect heartbeat (*5*, *6*, *7*).

The apparatus used to stimulate the heart preparation electrically consisted of the well-known student-type inductorium, key, signal magnet, dry cells, and a specially made pair of microelectrodes with tungsten tips. The signal magnet was included in the primary circuit and placed so that its lever would intercept the light passing through the end of the camera slit upon closure of the key of the primary circuit. Thus the shadow of the signal magnet appeared in the mechanocardiograms as lower marginal bands during the times the key of the primary circuit was closed. When single induced shocks were used they were applied to the tissue at times indicated by the beginning and the end of the marginal band. When the inductorium was so wired as to supply tetanizing shocks they were applied to the tissue in rapid succession throughout the duration of the period indicated by the marginal band. The time record, consisting of lines extending completely across the photographic paper, were made by a Lieb watch timer, the lever of which was made to cast a shadow across the entire camera slit at determined time intervals (1 second in these experiments).

The complete mechanocardiographic set-up of apparatus was essentially the same as that previously described (*4*). The electrocardiographic camera, however, has been remodeled to yield paper speeds ranging from about 0.5 to about 100 mm. per second. In order to adapt intensity of light to paper speed, the beam of light was passed through suitable filters. The records were made upon a roll of bromide photographic paper. Most of the experiments were

performed at room temperature, which was maintained fairly constant and usually at a temperature lying within the range 25° to 30° C.

Unless otherwise stated, the lever contact was with the dorsal diaphragm immediately over the cardiac tube. As the cardiac-muscle fibers contracted, the dorsal diaphgram and lever attachment were lowered and the lever moved so as to produce an upward deflection of the mechano-cardiographic curve and, conversely, when the diaphragm was raised the curve was depressed. Similarly, upward or downward deflections of the lever shadow on the photographic paper were produced when the diaphragm was lowered or raised by any other cause, as for example by movement of the preparation resulting from contractions of the dorsal body muscles. The position of the electrodes was changed from time to time as indicated in the legends of the illustrations and under the heading Results. The stimuli applied consisted of single induced shocks, induced shocks repeated by opening and closing the key of the primary circuit by hand, and series of induced tetanizing shocks applied at a rate determined by the vibrator of the inductorium. Because of current leakage through the saline from one electrode to another, a rather strong stimulating current was employed.

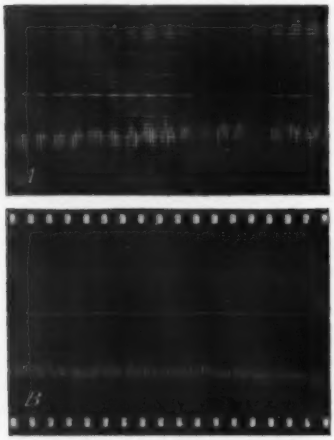

FIGURE 1.—*A*, Mechanocardiogram from a completely isolated fourth abdominal segment (preparation D) perfused with the oxygenated magnesium saline. Room temperature 29° C.; record taken 105 minutes after beginning of perfusion; lever over heart; electrodes in place on distal extremity of alary-muscle group; paper speed about 0.5 mm. per second; time markings in seconds; no electrical stimuli. *B*, Similar record from an incompletely isolated third abdominal segment (preparation C). Room temperature and perfusion as in *A*; preparation 105 minutes old; lever over heart; no electrodes.

All the mechanocardiograms obtained in these experiments are to be read from left to right.

RESULTS

EXTRASYSTOLES AND SUMMATION OF CONTRACTIONS

Figure 1, *A*, shows the regularity in rate and amplitude of heart contraction when the completely isolated segment (preparation D) was perfused continuously with the magnesium saline solution. *A* and *B* (preparation C) are parts of records taken at low paper speed in different experiments after each of the preparations had been perfused for about 105 minutes.

The results of applying single and repeated faradic stimuli to the completely isolated fourth abdominal segment (preparation D) are shown in figures 2, 3, and 4. Figure 2 (*A*, *a*; *B*, *a*) shows the extrasystoles produced by stimulating with single induced shocks during diastasis (the rest period of the cardiac cycle). These extrasystoles are a little greater than the spontaneous contractions (fig. 2, *A*, *c* and *d*; *B*, *c* and *d*).

In figure 3 the extrasystoles at *A*, *a* and *b*, and at *B*, *a*, were produced by single shocks applied at different times (*A*, *c* and *d* and *B*, *b*)

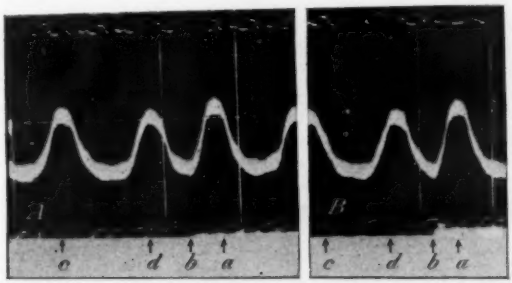

FIGURE 2.—Mechanocardiograms from a completely isolated fourth abdominal segment (preparation D), 145 minutes old. Room temperature 29° C.; perfusion with magnesium saline; lever over heart; electrodes over lateral extremity of alary-muscle group; time markings in seconds; and stimuli single induced shocks. *A*, Extrasystole at *a* produced by stimulus applied during late diastasis at *b*; *c* and *d* are spontaneous contractions. *B*, Extrasystole at *a* produced by stimulus applied during early diastasis at *b*; *c* and *d* are spontaneous contractions.

during diastole (relaxation period). The summation effect in *B* is greater than that in *A*. In figures 2 and 3 the electrodes were at the lateral extremity of an alary-muscle group.

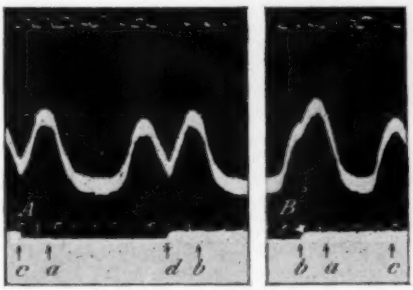

FIGURE 3.—Mechanocardiograms from a fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; time markings in seconds; lever over heart; electrodes over lateral extremity of alary-muscle group; single induced shocks applied. *A*, Extrasystoles at *a* and *b* caused by single shocks applied during late diastole at *c* and *d*, respectively. *B*, Extrasystole at *a* caused by a single shock applied at *b* during the beginning of the diastole of a spontaneous beat; *c* is a spontaneous contraction.

Similarly figure 4 shows the effect of applying single shocks (electrodes medially over an alary-muscle group) at different times during systole (contraction period). Apparent summation effects are produced. In figure 4, *B*, *d*, summation at the height of contraction is very evident. The shock applied at *A*, *c*, may have fallen during the refractory period, which would account for the contraction height being more nearly that of the spontaneous contractions *e* and *f*.

Figure 5 shows the production of extrasystoles by single induced shocks applied during late and early diastole to an abdominal heart preparation (type B) when the electrodes were on the dorsal diaphragm over the heart in the fifth abdominal segment and the lever over the heart in the fourth abdominal segment. The strength of the stimulus was not sufficient to cause interfering contractions of the body muscles. Summations of contractions are

shown, particularly at *f* and *h*, when the shocks *e* and *g* fell at the height of the contraction curve.

COMPENSATORY PAUSE

No recognizable compensatory pauses have been observed to follow the extrasystoles produced in these experiments.

TETANUS OF THE HEART

Figure 6 shows the result of applying to the completely isolated single fourth abdominal segment (preparation D) a series of single

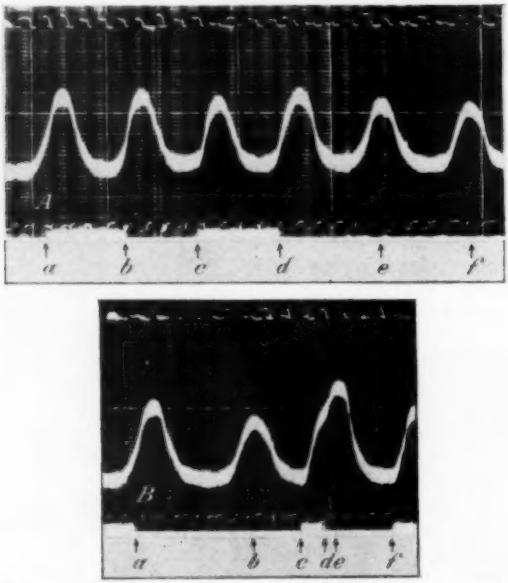

FIGURE 4.—Mechanocardiograms from a fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; lever over heart; electrodes in medial position over one alary-muscle group; single induced shocks applied; time in seconds. *A*, Single induced shocks were applied during systole at *a*, *b*, and *d*. The shock at *c* fell either at the end of diastasis or at the beginning of systole; *e* and *f* are spontaneous beats. *B*, Shocks *a* and *d* fall respectively in early and very late systole; *c* and *f* fall during diastasis; *b* is a spontaneous contraction; *e* is a marked summation of contractions.

induced shocks at gradually decreasing time intervals when both the lever and the electrodes are in contact with the dorsal diaphragm over the cardiac tube. *B* is a continuation of *A*, and *C* of *B*. At figure 6, *A*, *d*, summation effects appear and increase until, at *e*, a complete tetanus is developed, which persists until the cessation of stimuli at *B*, *f*. In *B*, between *f* and *g*, there occurs a rapid relaxation, followed by a quick appearance of spontaneous rhythm in which the systoles appear to increase gradually in magnitude as further apparent relaxation occurs. At *C*, *l*, complete recovery has apparently been made.

In the same way, in figure 7, *A* and *B* show cardiac tetanus produced by similarly applying a series of single induced shocks to the abdominal heart preparation (type B) when the lever was on the dorsal diaphragm over the heart in the fifth abdominal segment and the electrodes were over the lateral extremity of the insect's left alary-muscle group. Cardiac tetanus begins at *A*, *e*, lasts to the cessation of stimulation at *B*, *f*, and is followed by rapid relaxation from *f* to *g*. This is followed by a period of inhibition, broken by the spontaneous contractions *h*, *i*, and *j*, of increasing magnitude. Complete recovery is evident at *j*. The record of tetanic contraction of the heart, seen from *A*, *e*, to *B*, *f*, is not a smooth curve, as in figure 6, A, *e*, to *B*, *f*, but has superimposed upon it a series of irregularities the

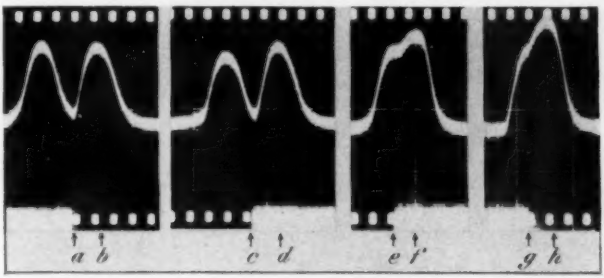

FIGURE 5.—Mechanocardiogram from an isolated abdominal preparation (type B). Room temperature 25° C.; heart preparation kept moist with 0.11 Levy's saline; lever over heart in fourth abdominal segment; electrodes over heart in fifth abdominal segment; time in seconds. Single induced stimuli applied at *a*, *c*, *e*, and *g* produced extrasystoles *b*, *d*, *f*, and *h*, respectively. The shocks were applied during late and early diastole.

larger of which are produced by interfering contractions of the dorsal body muscles given in response to the applied series of stimuli. The minor variations are like those seen in *A*, *a* to *b*, and were caused by mechanical vibrations of the apparatus that had nothing to do with tissue response.

In figure 7, *C*, is shown the first part of a cardiac tetanus similarly produced by stimulating an entire isolated heart preparation (type A). The marked interference of body-muscle contractions with the record of cardiac tetanus is shown.

STAIRCASE EFFECT

Apparent staircase phenomena have been observed, especially when spontaneous cardiac rhythm begins after the temporary inhibition following cardiac tetanus (fig. 6, *B*, *C*, and fig. 7, *B*), and when heart preparations begin spontaneous contractions after certain other periods of diastolic standstill not associated with cardiac tetanus.

REFRACTORY PERIOD

Results of the application of stimuli during various parts of systole (fig. 4) seem to indicate that the stimuli were effective during the greater portion of the contraction period and therefore, if the absolute refractory period occurs, it is confined to at least the early part of systole.

MECHANICAL STIMULATION OF THE HEART

Microscopic observations showed that when the dorsal diaphragm of an isolated heart preparation was mechanically stimulated in the region of the cardiac tube by touching it firmly (but without injury to the tissues) with the point of a dissecting needle, the response of the cardiac tube was a more or less sustained contraction. The con-

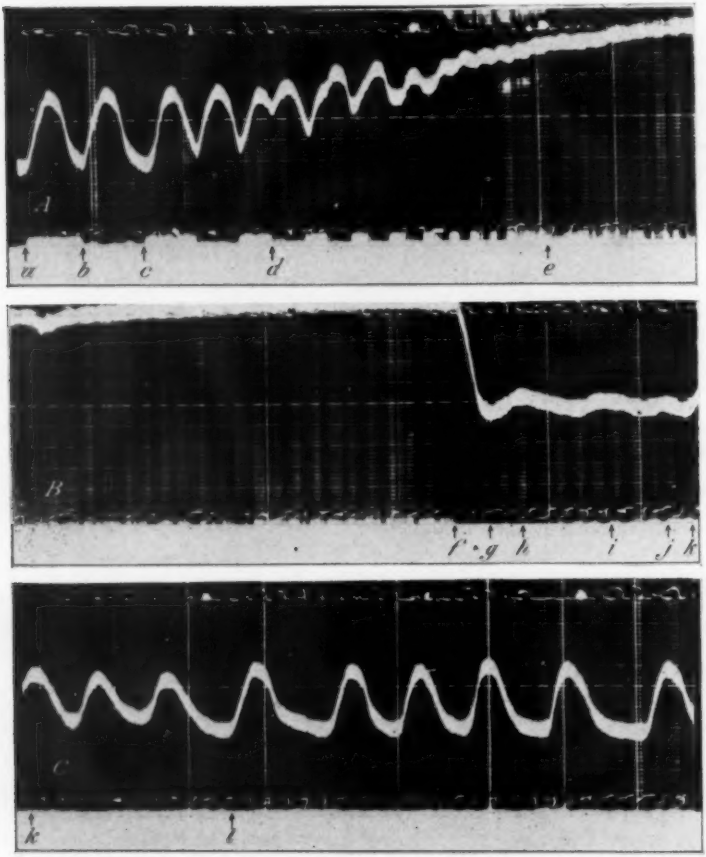

FIGURE 6.—Mechanocardiogram from a completely isolated fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; lever and electrodes over heart; time in seconds. *A*, Effects of a series of single induction shocks applied with decreasing time intervals at *a*, *b*, *c*, *d*, and so on. At *e*, complete tetanus is produced. *B*, Continuation of *A*. Stimuli cease to be applied at *f*, and relaxation is completed at *g*; at *h*, *i*, and *j*, incomplete spontaneous beats occur; *k* is part of the first beat *k* shown in *C*. *C*, Continuation of *B*. At *l*, recovery is complete.

traction did not always involve the entire cardiac tube but often was confined to the cardiac regions close to the point of stimulation. This localized contraction has been observed to persist while the spon-

taneous rhythm of other regions of the heart continued without interruption. Whether the contraction was of the nature of a cardiac tetanus, a cardiac contracture, or a different type of response is not known.

In these experiments, isolated heart preparations have often temporarily ceased to exhibit spontaneous rhythm apparently because of mechanical stimuli applied in the process of adjusting the contact

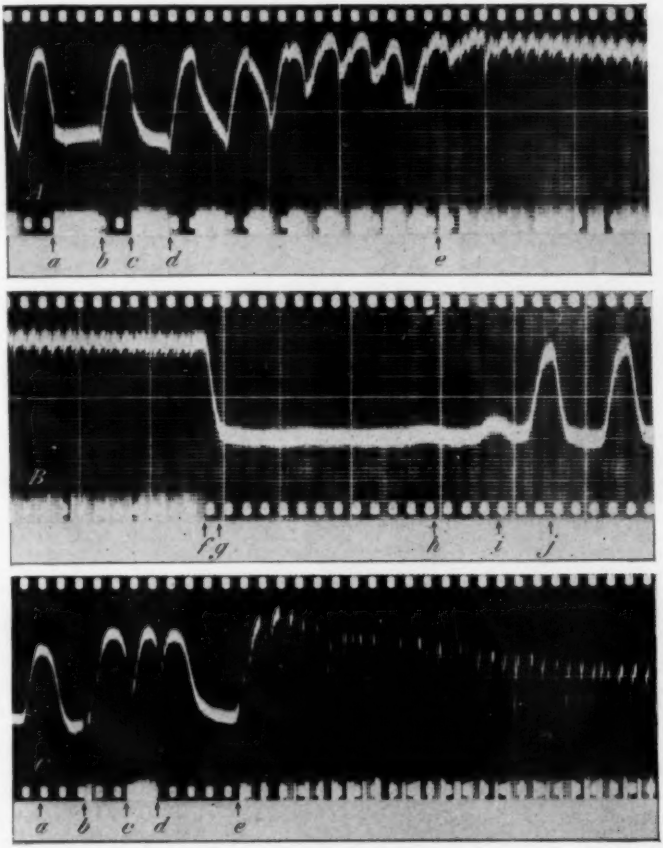

FIGURE 7.—*A* and *B*, Mechanocardiogram from an isolated abdominal heart preparation (type B). Room temperature, 25° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode on distal extremity of insect's left fifth abdominal alary-muscle group; time in seconds. *B* is a continuation of *A*. Single induced shocks applied at *a*, *b*, *c*, and so on, with decreasing intervals. At *e*, complete cardiac tetanus is produced, but the curve is disturbed by dorsal body-muscle contractions. The minor vibrations (as from *a* to *b*) are mechanical and have nothing to do with tissue response. *C*, Mechanocardiogram from an entire heart preparation (type A). Single induced shocks applied at *a*, *b*, *c*, and so on, and cardiac tetanus produced at *e*. The tetanus curve is disturbed by contractions of dorsal body muscles. Time in seconds.

163005—39——4

between lever and dorsal diaphragm. Under these conditions the cardiac response was very probably the same as the response observed to follow noninjurious mechanical irritation with a dissecting needle.

CONTRACTIONS OF THE DORSAL BODY MUSCLES

Figure 8, *A*, shows the effect of repeatedly stimulating an isolated abdominal heart preparation (type B) kept moist with Lévy's saline, when the lever contact was over the heart in the fifth abdominal segment and the electrodes were on lateral extremities of a fifth abdominal alary-muscle group. The paper speed was low. The downward trend of the contractions shown in *A* between *c* and *d* was caused by interfering body-muscle contractions. The single shocks applied at *a*

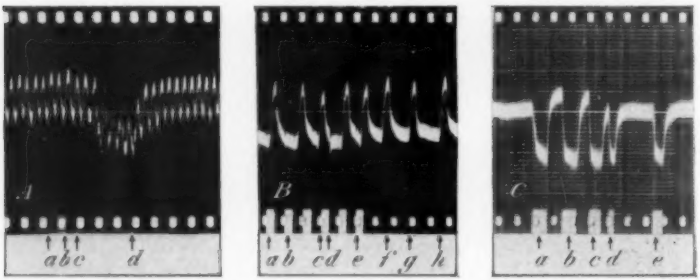

FIGURE 8.—*A* and *B*, Mechanocardiograms from an isolated abdominal heart preparation (type B). Room temperature 29° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode over lateral extremity of alary-muscle group in fifth abdominal segment. *A*, Single induced shocks at *a* and *b*, and a series of single shocks applied between *c* and *d*. *B*, Single induced shocks applied at *a*, *b*, and so on to *e*, with make shock applied at *c* and break shock applied at *d*, and so on. The disturbing effects of dorsal body-muscle contractions are shown. At *f*, *g*, and *h*, are undisturbed spontaneous beats. The stimulating current did not affect the heart, which continued to contract spontaneously. *C*, Record from same preparation after the heart had been severed longitudinally and the fourth, fifth, and sixth left alary-muscle groups removed. Lever medially and electrodes laterally in region where left alary-muscle group had been. Induced tetanizing shocks applied at *a*, *b*, and so on to *e*. Contractions of the dorsal body muscles depressed the curve during stimulation.

and *b* produced no marked effects in this record made with a low paper speed. In figure 8, *B*, are shown the effects of applying single induced shocks to the same preparation at different times in the cardiac cycle when greater paper speed was used. The interference of body-muscle contractions with the mechanocardiogram at the times of stimulation is evident. All the cardiac contractions are spontaneous, and those at *f*, *g*, and *h* exhibit no body-muscle effects.

The dorsal diaphragm and the cardiac tube of this preparation were then severed with a safety-razor blade and the fourth, fifth, and sixth left alary-muscle groups and associated dorsal diaphragm removed. The lever and electrodes were then placed in contact with the exposed dorsal body muscles of the left side, the lever medially and the electrodes laterally. The record thereupon obtained by faradic tetanizing stimulation is shown in figure 8, *C*. The stimuli, applied at *a*, *b*, *c*, *d*, and *e*, resulted in marked depressions of the otherwise straight-line

record. After the removal of the entire heart and the right alary-muscle groups, stimulation gave results similar to those shown in figure 8, *C*.

Figure 9 shows distortions of the mechanocardiogram caused by dorsal body-muscle contractions made in response to single induced

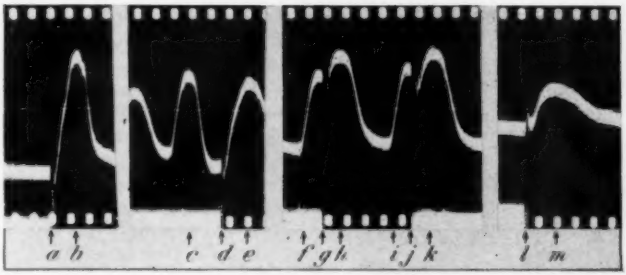

FIGURE 9.—Mechanocardiograms from a whole-heart preparation (type A). Lever and electrodes in an abdominal segment. Single induced shocks applied at *a*, *d*, *g*, *j*, and *l*. At *b*, *e*, *h*, *k*, and *m*, extrasystoles were thus produced. At *h* and *k*, summation effects are recorded. At the time of the stimulus distortions of the curve by dorsal body-muscle contractions can be seen. The upward deflection, at *l*, is unusual; dorsal body-muscle deflections were usually downward. Time in seconds.

shocks applied to the whole heart preparation (type A). Lever and electrode contacts were in an abdominal segment. The contractions *b* and *e* were given in response to stimuli applied at *a* and *d*, respectively, and were preceded by interfering body-muscle contractions

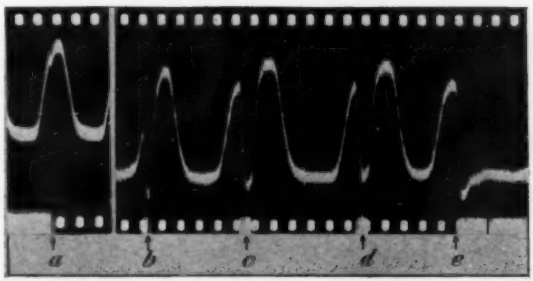

FIGURE 10.—Mechanocardiograms from an isolated abdominal heart preparation (type B). Room temperature 25° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode over distal extremity of insect's left fifth abdominal alary-muscle group; time in seconds. At *a*, a single induction shock was applied. At *b*, *c*, and *d*, short tetanizing stimuli were applied. At *e*, a more prolonged tetanizing stimulation was begun. The marked effects of dorsal body-muscle contractions on the mechanocardiograms are shown.

whose records somewhat resemble presystolic notches. Similar distortions, however, occurred during other parts of the cardiac cycle at *g*, *j*, and *l*. Usually the body-muscle contractions produced depressions of the curve, but at *l*, the deflection was upward.

Figure 10 shows similar results of applying short tetanizing stimuli to an abdominal heart preparation (type B). The lever was over the heart in the fifth abdominal segment, and the electrodes were over the lateral extremities of the left alary-muscle group of the same segment. The very small vibrations were mechanical and had nothing to do with tissue response. Marked disturbances of the heartbeat record are shown.

Figure 11 shows depressions of an otherwise straight-line record obtained from an incompletely isolated abdominal segment (preparation C) from which the heart and both alary-muscle groups had been removed. The lever was in the region where the heart had been, and the electrodes were placed laterally where one of the alary-muscle groups had been located. Series of single induced shocks were applied between *a* and *b* and between *c* and *d*. During stimulation the otherwise straight-line record was depressed by contractions of the dorsal body muscles. The external dorsal body muscles are considered to be responsible for this movement.

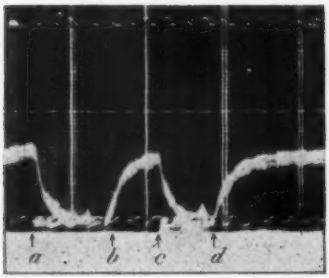

FIGURE 11.—Mechanocardiogram from an incompletely isolated third abdominal segment (preparation C) about 5 hours old. Room temperature 29° C.; preparation perfused with the magnesium saline; time in seconds. This record was obtained after cardiac tube and both alary-muscle groups had been removed. Lever on midregion where cardiac tube had been; electrodes lateral to lever, in region where one alary-muscle group had been. From *a* to *b* and from *c* to *d*, a series of single induced shocks were rapidly applied. The contractions of the dorsal body muscles depressed the curve.

When the completely isolated third abdominal segment (preparation D) from which the heart and both alary-muscle groups had been removed was similarly stimulated with induced shocks, applied either singly or in rapid succession, or with tetanizing stimuli, no appreciable effect of body-muscle contraction upon the otherwise straight-line record was observed.

SUMMATION OF STIMULI

It was observed that when slightly subminimal single shocks were rapidly applied by manipulating the key of the primary circuit or when tetanizing stimuli of subminimal strength were applied, contractile responses of the heart and of the dorsal body muscles could be obtained. This indicates that summation of stimuli occurred in these muscles.

ALARY MUSCLES

No convincing evidence was obtained that the application of single induced shocks or of tetanizing stimuli to the alary-muscle fibers caused them to give responsive contractions. When the alary muscles were stimulated while they were under low-power microscopic observation they were not observed to respond with visible contraction either in the form of twitches or of visible tonus changes.

DISCUSSION

It is generally recognized [3] that the vertebrate heart (especially frog and mammalian ventricles) can respond to applied stimuli with extrasystoles, particularly when the stimuli are strong and are applied during late diastole or during diastasis. Because it possesses an absolute refractory period that persists throughout the systole and a relative refractory period that extends into at least the earlier portion of diastole, vertebrate cardiac muscle is not responsive to stimulation while it is contracting and responds to only unusually strong stimuli while it is relaxing, especially during early diastole. For the same reasons and because it exists in syncytial form, vertebrate cardiac muscle does not normally exhibit well-defined summation of contractions in response to single shocks or complete tetanic contractions when stimulated with tetanizing shocks. It can display staircase phenomena, compensatory pauses, and response to mechanical as well as to electrical and other stimuli. Its response to mechanical stimulation, applied by touching the ventricle with the point of a needle, appears as a twitch, not as a sustained contraction.

Very little has been known of the responses to electrical stimulation of the insect heart, especially the isolated insect-heart preparation. From the results of these experiments it is evident that in the perfused isolated-heart preparation of *Periplaneta americana* the cardiac responses differ considerably from the responses usually reported to be given by vertebrate hearts. The cockroach heart can respond to single induced shocks with extrasystoles during both diastasis and diastole. The extrasystolic contractions are more or less summated, the degree of summation being greatest at the height of the contraction curve, that is, at the very beginning of diastole and perhaps at the very end of systole. These results would seem to indicate either (1) that the cockroach cardiac muscle does not react syncytially as does vertebrate cardiac muscle, but behaves more like skeletal muscle, in which the individual fibers, but not the muscle as a whole, give the all-or-none response, or (2) that the cockroach cardiac muscle functions syncytially but fails to obey the all-or-none law and gives graded contractions. They also indicate that at the height of contraction and in early diastole the insect cardiac muscle is not in an absolute refractory state and probably is not in a marked relative refractory state. If, furthermore, the slight increase in contraction height resulting from the application of a single shock during the early, middle, or late portion of systole is to be interpreted as a summation of contractions of the cardiac-muscle fibers [4] given in response to the stimulus, it would seem that the insect cardiac muscle fails to exhibit absolute refractoriness throughout all except perhaps the very early part of systole.

The cockroach heart gives a well-defined tetanus in response not only to tetanizing stimuli but also to single shocks rapidly applied by opening and closing the key of the primary circuit by hand. In this response the cockroach heart differs from the vertebrate heart. The

[3] See various textbooks, manuals, and reviews of vertebrate physiology for prevailing opinions as to the usual characteristics of vertebrate hearts and cardiac muscle, for example, books by Howell, Starling, Bayliss, MacLeod, and others.

[4] If the increased contraction height of the curve is not caused by additional cardiac-muscle contraction, it seems that it would have to result from the alary-muscle fibers responding to the stimulus with relaxation, since contractions of the alary-muscle fibers would be expected to depress, and the contractions of the dorsal body muscles have been found nearly always to depress the curve of heartbeat. This would be contrary to the way muscle fibers are now known to respond to stimuli.

cardiac tetanus thus obtained serves as additional evidence that at the height of the contraction curve the insect cardiac muscle is not in a state of absolute refractoriness.

The cockroach heart also differs from the vertebrate heart in that no recognizable compensatory pauses have been found to follow extrasystoles. Although occasionally certain extrasystoles have been followed by diastatic periods somewhat longer than those preceding the same extrasystoles, the observed increase of diastasis is not significantly great when the variations in duration of diastasis that commonly occur spontaneously are considered. The lack of occurrence of compensatory pauses by the cockroach heart suggests that stimulating this heart is analogous to stimulation not of the ventricle but rather of the sinus venosus, for example, of the frog heart.[5] If this analogy is correct, it implies that in stimulating the cockroach heart, as was done in these experiments, the stimuli act upon something corresponding to pacemaker tissue or to a tissue that sets its own pace. This would not be unexpected where, as in most of these experiments, a strong stimulus is applied directly to the cardiac muscle of an isolated segment of the heart mechanism.

Comparison of the results obtained with the different types of isolated heart preparation used in these experiments shows that movement of the preparation resulting from contractions of the dorsal body muscles is a factor that must be brought into consideration when experiments are undertaken in which insect heart preparations are subjected to various stimuli, either electrically or by the application of certain dissolved substances.[6] It is evident that one way to eliminate interfering movements of the dorsal body muscles is to utilize preparation D (see Methods) in which the internal and external dorsal body muscles are either severed or torn from their insertions.

The movements caused by contractions of the dorsal body muscles may affect the mechanocardiogram in more than one way, but in these experiments the effect was nearly always to depress the record of heartbeat. With strong single shocks, sharp, rapid, momentary depressions at the time of stimulus are superimposed on the mechanocardiogram and, when occurring just before systole, may somewhat resemble the presystolic notch. This resemblance may be quite marked when the stimulus is a very short tetanizing current. The fact that through stimulation the body-muscle depressions can be made to occur at other times during the cardiac cycle than just prior to systole indicates the difference between these depressions and the spontaneously occurring presystolic notches. This is shown also by comparison of the forms of the body-muscle depressions and the presystolic notches. It is hardly to be expected that dorsal body-muscle contractions are the cause of the presystolic notch since this would have the improbable meaning that the dorsal body muscles contract rhythmically in exact synchronism with the heart rhythm. The origin of the presystolic notch, therefore, is probably to be sought among other causes.

[5] A compensatory pause often follows an extrasystole of the frog heart produced by stimulation of the ventricle. The pause is thought to be caused by the next excitatory impulse from the sinus venosus (pacemaker) reaching the ventricle when the latter is in the refractory state produced during the extrasystole. The ventricle has to wait for still another excitatory impulse to arrive from the pacemaker before it contracts. Stimuli applied to the sinus venosus (pacemaker), therefore, produce extrasystoles that are not followed by compensatory pauses.

[6] Since nicotine can produce contraction or contracture in skeletal muscle (2), its effect upon insect dorsal body muscle deserves consideration, particularly when high or toxic concentrations are used.

In none of these experiments has evidence been obtained that the applied stimuli produced recognizable contractions of the alary-muscle fibers. It is possible that the alary-muscle fibers contracted when stimulation caused interfering contractions of the dorsal body muscles, but when these interfering movements were not produced, the alary muscles exhibited no signs of undergoing contraction, even when the stimulating electrodes were over the alary-muscle fibers themselves and the stimulus was strong enough to cause contraction of the cardiac muscle. Neither did the alary-muscle fibers show any recognizable shortening when observed microscopically during stimulation. Although this evidence does not constitute proof that the alary-muscle fibers are incapable of contraction, it does render more probable the hypothesis that the alary-muscle fibers of *Periplaneta americana* tend to maintain a tonus and to apply a steady pull upon the wall of the cardiac tube rather than to produce diastole by rhythmic twitches given synchronously with the rhythm of the cardiac muscle. These facts would also indicate that even should contractions of the alary-muscle fibers occur during, and be undetected because of, interfering body-muscle contractions, the threshold of excitability of the alary-muscle fibers is considerably higher than that of the cardiac muscle in the same abdominal segment.

The failure to obtain recognizable contraction of the alary-muscle fibers in these experiments also lends weight to the hypothesis (*4*) that the presystolic notch results not from the rhythmic presystolic contraction of the alary-muscle fibers but probably from a presystolic increase of intracardiac hydrostatic pressure resulting from the contraction of the heart in some segment other than that from which the mechanocardiogram is being recorded.

These isolated heart preparations no doubt contained intrinsic nervous mechanisms (*5*) that probably have to do with cardiac regulation. What effect these structures had in the response of the heart to stimulation in these experiments is not known, but, since leakage of electrical current through the perfusion saline from one electrode to the other necessitated the use of strong stimuli, it was assumed that the cardiac, alary, and dorsal body muscles were subjected to direct stimulation.

SUMMARY AND CONCLUSIONS

Four different types of isolated heart preparations from the American cockroach (*Periplaneta americana* (L.)) have been electrically stimulated and mechanocardiograms of their responses recorded. The four types were: Type A, the whole isolated heart preparation, including both thoracic and abdominal portions; type B, the abdominal heart preparation; type C, the single abdominal segmental preparation, incompletely separated from the adjacent margin of the adjacent overlapping segment but completely separated from the adjacent overlapped segment; and type D, the single abdominal segment completely separated from both adjacent segments. The preparations were occasionally flooded or continuously perfused with saline solutions.

The applied stimuli consisted of single induced shocks, induced shocks rapidly repeated by opening and closing the key of the primary circuit by hand, and induced tetanizing shocks consisting of a series of

shocks applied at the rate determined by the vibrator of the inductorium.

The mechanocardiograms obtained show that the heart of this insect can respond to single shocks, applied during diastasis and diastole, with extrasystoles that are more or less summated, particularly during early diastole; that it gives an apparent summation response when the shock falls during systole; and that the extrasystoles thus obtained are not followed by compensatory pauses.

It is also shown that the heart can respond to tetanizing stimulation with a complete cardiac tetanus and can rapidly relax and quickly its original spontaneous rhythm after the cessation of stimulation. Posttetanic standstills often occur.

During recovery from posttetanic standstill and during recovery from standstill produced by certain other causes, the cockroach heart exhibits apparent staircase phenomena.

The responses of this heart to single shocks applied at different times in the cardiac cycle and to tetanizing stimuli show that this insect's cardiac muscle is not absolutely refractory during diastole, diastasis, and, probably, late systole. They also indicate that, if the absolute refractory period exists in this cardiac muscle, it is probably confined to early systole.

This insect heart can respond to mechanical stimulation with a more or less prolonged contraction, which may be localized in that part of the heart tube lying near the point of stimulation.

Contractions of the dorsal body muscles may cause variations to be superimposed on the heartbeat record. The variations may stimulate the presystolic notches when they are caused by shocks eliciting extrasystoles or when they happen to fall immediately prior to systoles.

The heart of this insect differs from the vertebrate heart (particularly frog and mammalian ventricles) in its ability to respond to stimulation with summated extrasystolic contractions, in its failure to exhibit compensatory pauses, in its apparently shorter absolute refractory period, and in its ability to respond to a mechanical stimulus with a more or less prolonged contraction.

Stimulation of the alary-muscle fibers (of the heart) with strong single, repeated, or tetanizing shocks yielded no evidence that they responded with recognizable contractions in the form of either twitches, tetanus, or tonus changes. These negative results lend weight to the hypothesis that cardiac dilation during diastole is not produced by rhythmic contractions of the alary muscles but rather that the latter tend to exert a steady tension on the cardiac walls. They also support the view that the presystolic notch is produced not by presystolic contractions of the alary-muscle fibers but by some other agency (probably increases of intracardiac hydrostatic pressure).

Interfering contractions of the internal and external dorsal body muscles may interfere with mechanocardiograms obtained from heart preparations of types A, B, and C. These can be eliminated by using the type D preparation.

LITERATURE CITED

(1) BABERS, FRANK H.
1938. AN ANALYSIS OF THE BLOOD OF THE SIXTH-INSTAR SOUTHERN ARMYWORM (PRODENIA ERIDANIA). Jour. Agr. Research 57: 697–706.
(2) LANGLEY, J. N.
1913. THE PROTRACTED CONTRACTION OF MUSCLE CAUSED BY NICOTINE AND OTHER SUBSTANCES CHIEFLY IN RELATION TO THE RECTUS ABDOMINIS MUSCLE OF THE FROG. Jour. Physiol. 47: [159]–195, illus.
(3) LEVY, R.
1928. FONCTIONNEMENT DU VAISSEAU DORSAL DE LA LARVE DE MOUCHE EN MILIEU ARTIFICIEL. Comp. Rend. Soc. Biol. [Paris] 99: 1482–1485.
(4) YEAGER, J. FRANKLIN.
1938. MECHANOCARDIOGRAPHIC METHOD OF RECORDING INSECT CARDIAC ACTIVITY, WITH REFERENCE TO THE EFFECT OF NICOTINE ON ISOLATED HEART PREPARATIONS OF PERIPLANETA AMERICANA Jour. Agr. Research 56: 267–276, illus.
(5) ——— and GAHAN, J. B.
1937. EFFECTS OF THE ALKALOID NICOTINE ON THE RHYTHMICITY OF ISOLATED HEART PREPARATIONS FROM PERIPLANETA AMERICANA AND PRODENIA ERIDANIA. Jour. Agr. Research 55: 1–19, illus.
(6) ——— and HAGER, ANNA.
1934. ON THE RATES OF CONTRACTION OF THE ISOLATED HEART AND MALPIGHIAN TUBE OF THE INSECT, PERIPLANETA ORIENTALIS. METHOD. Iowa State Col. Jour. Sci. 8: 391–395, illus.
(7) ——— HAGER, ANNA, and STRALEY, J. M.
1935. SOME PHYSIOLOGICAL EFFECTS OF CERTAIN ALIPHATIC THIOCYANATES ON THE ISOLATED HEART PREPARATION FROM THE ROACH, BLATTA ORIENTALIS. Ann. Ent. Soc. Amer. 28: 256–264, illus.

JELLYING AND CRYSTALLIZATION OF SIRUPS MADE FROM DIFFERENT PARTS OF THE SORGO STALK AT DIFFERENT STAGES OF MATURITY[1]

By Emil K. Ventre, *chemist*, S. Byall, *assistant chemist*, and C. F. Walton, Jr., *senior chemist, Carbohydrate Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture*

EARLIER WORK

The fact that starch is present in sorgo juices has been known for many years. In 1858 Gilbee (*3*)[2] was granted a British patent on a process for eliminating organic impurities from the juices of saccharriferous plants by the use of alcohol. In 1864 Joulie (*5*), in recommending a method of clarification by the use of alcohol, mentioned that the alcohol precipitate contained starchy materials, and suggested conversion to sugar for production of alcohol. Sylvester (*10*) found the starch granules in sorgo juice to be from one eight-thousandth to one six-thousandth of an inch in diameter, as compared with one five-hundredth to one four-hundredth of an inch for granules of potato starch. Hutchinson (*9*), in discussing the distribution of starch in the sorgo stalk, reported that the iodine test showed no starch in green canes. If any blue color was present it was obscured by an intense brown coloration. With well-matured canes iodine gave an intensely blue color toward the top, which decreased in intensity toward the butt. At the beginning of maturity, as indicated by the presence of sucrose in the lower part of the stalk, more starch was found in the butt than in the top. Janssen, McClelland, and Metzger (*4*) in reporting analyses of sorgo juices from the different internodes of the Honey variety stated that starch occurs throughout the internodes of the stalk, although it is highest in the middle internodes.

Sherwood (*6*, *7*) reported the starch content of the juices of 15 varieties of sorgo and proved that starch is the cause of jellying or clabbering of sorgo sirups. Willaman and Davison (*11*), in studying the jellying of sorgo sirups, found that 0.32 to 1.15 percent of starch is present in normal sirups and 1.17 to 3.36 percent in sirups that jelly. Walton and Ventre[3] found that starch is responsible for slow boiling and scorching in the evaporator, and devised a farm-scale method for preventing this condition.

Many investigators have attempted to develop a practicable process for utilizing sorgo as a commercial source of sugar (sucrose). Among those who also studied the dextrose and levulose content of the stalk were Berthelot and Trannoy (*2*), who reported analyses of sorgo juices at eight stages of plant maturity from August 10 to November 30. At each stage of maturity the juices contained from approximately

[1] Received for publication March 17, 1939.

[2] Italic numbers in parentheses refer to Literature Cited, p. 150.

[3] Walton, C. F., Jr., Ventre, E. K., and Byall, S. How to prevent slow boiling, scorching, clabbering, and sugaring of sorghum sirup. U. S. Bur. Chem. and Soils Cir. 6 pp. 1935. [Mimeographed.]

two to four times as much dextrose as levulose, and at the two earliest stages the dextrose content exceeded the sucrose content. It appears, however, that these earlier investigators did not study the crystallization characteristics of sirups made from the stalk as a whole in comparison with those of sirups made from different parts of the stalk. Moreover, it must have been assumed that the sugar content of the sorgo stalk is similar to that of sugarcane. No report has been found of dextrose crystallization in sorgo sirups.

On the whole the literature indicates that earlier investigators made little attempt to correlate composition of the juice and quality of the sirup, especially the effect of the content of starch, sucrose, and dextrose in the juice on the composition and properties of the resulting sirups.

In the manufacture of sorgo sirups by the customary methods, the effect on the starch and sucrose of continued heating in a slightly acid solution should produce a somewhat greater proportion of dextrose in the sirups than in the juices. Since both the content of starch and sugars in different parts of the stalk and the effect of maturity on the composition of the juice must be considered, it is obvious that farm-made sorgo sirups present a special case of the system sucrose, dextrose, and levulose in a relatively impure solution containing also varying proportions of starch and its degradation products produced by the action of heat and acidity during open evaporation. The purpose of the present investigation was to determine the effect of the sugar and starch content of sirups in relation to crystallization and jellying. Sirups made from different parts of the stalk at different stages of maturity were compared with those made from the whole stalk. Inasmuch as the sirups were made from different portions of the same stalk, an opportunity was afforded to study jellying and crystallization under uniform agronomic conditions.

EXPERIMENTAL PROCEDURE

In continuation of previous work by the authors (*12*) an investigation was conducted in cooperation with the Mississippi Agricultural Experiment Station [4], the four varieties of sorgo (*Holcus sorghum* var. *saccharatus*) most used for sirup production in that section being selected for study. These varieties, designated by the names locally assigned to them, had the following varietal characteristics:

Hodo.—A large-barrel, long-stalk, late-maturing variety.

Iceberg.—A small-barrel, short-stalk, early-maturing variety.

Honey.—A small-barrel, medium-height stalk, intermediate-maturing, sprangle-top variety.

Gooseneck.—A medium-barrel, medium-height stalk, intermediate-maturing variety.

The stages of maturity were determined by the average maturity of the seed heads, described as follows:

Milk stage.—When the seed heads were out of the sheath and well formed, and the contents when pressed out had the consistence of milk.

Dough-to-ripe stage.—When the contents pressed from the seed heads had the consistence of stiff dough, the stage just preceding the hard-dough stage.

Dead-ripe stage.—When the contents of the seed heads were solid, and the glumes were wide open and dry.

[4] The authors wish to express their appreciation for assistance given by the late J. R. Ricks, director, and by W. R. Perkins, formerly assistant director, Mississippi Agricultural Experiment Station, in supplying working facilities and in connection with the agronomic work of this investigation.

When the sorgo had reached the selected stage of maturity, the stalks were cut even with the surface of the ground and stripped of leaves. The seed head and peduncle were removed, and the stalks divided at the nodes. The internodes were numbered consecutively from the peduncle and segregated in batches according to number. Each batch was ground separately in a horse-driven farm mill, being passed through the mill twice in order to extract as much juice as possible with this type of milling equipment.

A whole-stalk sample of each variety at each stage of maturity was used for comparison. An aliquot portion of the stalks was selected and stripped clean, after which the seed heads and peduncle were removed. This sample was subjected to exactly the same treatment as the various batches of internodes.

The juices expressed separately from each of two consecutively numbered batches of internodes were combined, and this juice was made into sirup for comparison with the sirup made from the whole stalk. The experimental method developed by this Division (*8*) for producing farm-made sirups was used in making all the sirups. Samples of the sirups were taken in triplicate and sealed while hot in 4-ounce, screw-top, glass jars. One sample was used for analysis; the other two were stored in a constant-temperature room at 20° C. for about 2 years. The sirups were made and analyzed in the fall of 1935, and the final examination for jellying and crystallization was made in February 1938.

The analytical determinations were made as follows:

Total solids.—Determined directly on the sirups at 20° C. by the use of the Zeiss sugar refractometer.

Sucrose.—Determined by the Association of Official Agricultural Chemists method of double polarization (*1*), invertase being used as the inverting reagent.

Reducing sugars.—Determined by the Munson and Walker method (*1*) and calculated in terms of invert sugar.

Starch was determined by the authors' modification of the official A. O. A. C. method (*1*) of starch determination in the presence of interfering polysaccharides. This method may be described briefly as follows:

For the malt reagent grind well-cleaned, new barley malt of high diastase activity and prepare an infusion of this freshly ground malt, just before it is to be used, by digesting 5 gm. with 100 cc. of water at room temperature for 2 hours, or for 20 minutes if the mixture can be stirred by an electric mixer. Filter to obtain a clear extract, if necessary returning the first portions of the filtrate to the filter. Mix the infusion well.

Wash a 15-gm. portion of the sirup into a beaker with approximately 300 cc. of 70-percent alcohol (by volume) and thoroughly mix by stirring.

Wash the precipitated starch free of sugar with additional 70-percent alcohol by decantation through an alundum crucible (porosity R. A. 360). Return the crucible to the beaker containing the washed starch precipitate and dry in an oven at 105° C. until free of alcohol. Then add 160 cc. of hot water and thoroughly gelatinize the starch at boiling temperature in a water bath. (At this point start a control to determine the quantity of dextrose derived from the malt reagent. This is done by adding 160 cc. of distilled water to a 400-cc. beaker and following the same procedure used for the starch determination.)

Cool to 50° C. or lower. Add 20 cc. of malt extract reagent and place in a temperature-controlled water bath. Keeping the mash thoroughly mixed, gradually raise the temperature to 70° in 20 to 30 minutes. Maintain at 70° for 30 minutes, stirring from time to time; then increase the temperature to 80° and keep it at that temperature for 10 minutes. Finally heat to the boiling point. Keep the mixture well stirred. Cool the contents of the beakers and the water bath to 55°. Add another 20 cc. of the malt-extract reagent, mix well, and hold at 55° for 1 hour, stirring about once every 10 minutes. At the termination of the digestion increase the temperature rapidly to above 80°.

Transfer to a 500-cc. volumetric flask and add 316 cc. of 95-percent alcohol, a little at a time, shaking between additions. Wash the crucible and beaker with hot water and use the washings to make up to the mark. Filter through a dry filter paper and evaporate the filtrate to a volume of 15 to 20 cc. or until free from alcohol.

Transfer the aqueous residue to a 200-cc. volumetric flask. Wash the beaker with hot water, using a rubber-tipped rod to recover any dextrin present, and add the washings to the volumetric flask. Allow it to cool, and then complete the volume to 200 cc. Transfer the contents to a suitable digestion flask, add 20 cc. of hydrochloric acid (specific gravity 1.125), made by diluting 68 cc. of strong acid (sp. gr. 1.19) to 100 cc., and connect the flask with a reflux condenser. Heat in a boiling water bath for 2½ hours Cool and transfer to a 250-cc. volumetric flask. Partly neutralize, while stirring, by adding 10 cc. of strong caustic soda solution (44 gm. NaOH per 100 cc. of H_2O), and complete neutralization with a little anhydrous Na_2CO_3. Cool to room temperature and make up to the 250-cc. mark.

Determine the dextrose in a 50-cc. aliquot. Correct the weight of dextrose obtained by subtracting the weight of dextrose obtained in a 50-cc. aliquot of the malt reagent control. Multiplying this value by 0.9 gives the weight of starch in the 50-cc. aliquot. This value $\times \frac{5}{18} \times 100 =$ the percentage of starch in the sirup.

EXPERIMENTAL RESULTS

Tables 1, 2, 3, and 4 give the sucrose, reducing sugars, and starch content of the sirups, and the calculated amounts of each in grams per 100 gm. of water as determined by the refractometer. The tables also show the ratio of sucrose to reducing sugars, the ratio of reducing sugars to sucrose, and the type of crystallization and jellying, if any.

TABLE 1.—*Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Gooseneck variety of sorgo at different stages of maturity*

Sirup No.	Internodes	Total solids [1]	Water [1]	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization	Starch			Jellying
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose		On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	
	Nos.	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*				*Percent*	*Grams*	*Grams*	
MILK STAGE																
1	1–2	79.30	20.70	16.20	20.43	78.26	52.45	66.13	253.38	0.30	3.23	Dextrose	0.39	0.50	1.91	Negative.
2	3–4	75.10	24.90	19.38	25.80	77.83	50.57	67.33	203.09	.38	2.61	do	.27	.36	1.08	Do.
3	5–6	78.60	21.40	21.95	27.92	102.57	51.90	66.02	242.52	.42	2.36	do	.23	.30	1.10	Do.
4	7–8	77.90	22.10	19.38	24.87	87.69	54.05	69.37	244.57	.35	2.78	do	.19	.25	.88	Do.
5	9–10	76.50	23.50	5.75	7.52	24.46	65.95	86.20	280.63	.08	11.46	do	.16	.21	.70	Do.
6	11–12	74.10	25.90	4.24	5.72	16.37	64.50	87.03	249.03	.06	15.21	do	.07	.09	.27	Do
DOUGH-TO-RIPE STAGE																
7	1–2	73.70	26.30	34.37	46.63	130.68	29.80	40.43	113.30	1.15	0.86	Negative	0.44	0.60	1.68	Negative.
8	3–4	79.30	20.70	39.97	50.40	193.09	32.65	41.17	157.72	1.22	.81	Sucrose	.39	.50	1.91	Do
9	5–6	74.70	25.30	29.67	39.71	117.27	39.90	53.41	157.70	.74	1.34	Dextrose	.28	.38	1.12	Do.
10	7–8	72.10	27.90	20.14	27.93	72.18	48.24	66.90	172.90	.41	2.39	do	.16	.22	.56	Do.
11	9–10	76.70	23.30	16.65	21.71	71.45	56.11	72.12	240.81	.29	3.37	do	.09	.12	.39	Do.
12	11–12	78.30	21.70	16.50	21.07	76.03	56.63	72.32	260.96	.29	3.43	do	.08	.11	.39	Do.
13	[2]	75.50	24.50	33.46	44.31	136.57	36.80	48.74	150.20	.91	1.10	do	.20	.27	.83	Do.
DEAD-RIPE STAGE																
14	1–2	75.80	24.20	41.33	54.52	170.78	23.78	31.37	98.26	1.74	0.57	Negative	0.86	1.14	3.57	Jellied.
15	3–4	76.90	23.10	44.21	57.48	191.38	24.98	32.48	108.13	1.77	.56	do	.83	1.08	3.59	Do.
16	5–6	77.00	23.00	38.30	49.74	166.52	31.80	41.29	138.26	1.20	.83	Sucrose	.77	1.00	3.34	Do
17	7–8	75.70	24.30	33.15	43.75	136.41	35.89	47.36	147.69	.92	1.08	Negative	.57	.76	2.36	Do.
18	9–10	74.50	25.50	29.67	39.82	116.35	43.49	58.53	170.54	.68	1.46	Dextrose	.43	.58	1.69	Negative
19	11–12	72.70	7.30	24.83	34.15	90.95	40.81	56.13	149.49	.60	1.64	do	.42	.58	1.54	Do.
20	[2]	74.10	25.90	37.40	50.46	144.40	30.95	41.76	119.49	1.21	.83	Negative	.67	.91	2.60	Jellied.

[1] By refractometer.
[2] Whole stalk.

TABLE 2.—*Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Honey variety of sorgo at different stages of maturity*

Sirup No.	Internodes	Total solids [1]	Water [1]	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization	Starch			Jellying
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose		On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	
	Nos.	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*				*Percent*	*Grams*	*Grams*	
MILK STAGE																
21	1–2	77.60	22.40	24.07	31.03	107.45	38.80	50.01	173.21	0.62	1.61	Dextrose	0.82	1.06	3.67	Jellied.
22	3–4	80.20	19.80	27.40	34.18	138.38	45.55	56.82	230.05	.60	1.66	do	.65	.81	3.28	Do.
23	5–6	81.40	18.60	20.89	25.67	112.31	56.20	69.07	302.15	.37	2.69	do	.49	.61	2.67	Do.
24	7–8	73.50	26.50	13.02	17.72	49.13	57.55	78.33	217.17	.22	4.42	do	.47	.64	1.77	Negative.
25	9–10	71.60	28.40	2.88	4.02	10.14	65.75	91.82	231.51	.04	22.83	do	.39	.54	1.37	Do.
26	11–12	73.40	26.60	1.36	1.85	5.11	65.30	88.96	245.49	.02	48.01	do	.26	.35	.97	Do.
27	[2]	76.40	23.60	20.28	27.85	85.93	50.85	66.58	215.46	.40	2.50	do	.46	.60	1.94	Do.
DOUGH-TO-RIPE STAGE																
28	1–2	79.50	20.50	35.76	48.75	189.07	29.42	37.00	143.51	1.31	0.76	Negative	0.52	0.66	2.56	Jellied.
29	3–4	77.30	22.70	40.58	52.49	178.76	31.77	41.09	139.95	1.27	.78	Sucrose	.51	.66	2.25	Do.
30	5–6	75.90	24.10	34.82	45.87	144.48	38.20	50.32	158.50	.91	1.10	Dextrose	.66	.87	2.74	Do.
31	7–8	73.50	26.50	25.74	35.02	97.13	44.50	60.54	167.92	.57	1.72	do	.55	.75	2.07	Negative.
32	9–10	76.50	23.50	17.11	22.36	72.80	54.95	71.82	233.83	.31	3.21	do	.56	.73	2.37	Do.
33	11–12	72.60	27.40	10.60	14.60	38.68	56.15	77.33	204.93	.19	5.29	do	.40	.56	1.48	Do.
34	[2]	72.80	27.20	24.22	33.26	89.04	45.25	62.15	166.36	.53	1.86	do	.52	.71	1.90	Do.
DEAD-RIPE STAGE																
35	1–2	76.90	23.10	24.22	31.49	104.84	40.75	52.98	176.40	0.59	1.68	Negative	0.55	0.72	2.40	Jellied.
36	3–4	80.00	20.00	31.95	39.93	159.75	40.50	50.62	202.50	.79	1.26	do	.60	.75	3.00	Do.
37	5–6	71.30	28.70	17.87	25.06	62.26	50.02	70.14	174.28	.36	2.78	Dextrose	.48	.67	1.66	Negative.
38	7–8	73.30	26.70	22.41	30.57	83.93	48.10	65.61	180.15	.46	2.14	do	.74	1.01	2.77	Jellied.
39	9–10	71.20	28.80	5.30	7.44	18.40	62.75	88.12	217.88	.08	11.84	do	.66	.92	2.29	Do.
40	11–12	72.30	27.70	5.60	7.74	20.21	63.05	87.19	227.61	.08	11.26	do	.60	.83	2.16	Negative.
41	[2]	78.60	21.40	37.40	47.58	174.76	35.47	45.12	165.75	1.05	.95	Negative	.80	1.02	3.74	Jellied.

[1] By refractometer.
[2] Whole stalk.

TABLE 3.—*Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Iceberg variety of sorgo at different stages of maturity*

Sirup No.	Internodes	Total solids [1]	Water [1]	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization	Starch			Jellying
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose		On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	
				DOUGH-TO-RIPE STAGE												
	Nos.	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*				*Percent*	*Grams*	*Grams*	
42	1–2	81.90	18.10	52.38	63.95	289.39	21.55	26.31	119.06	2.43	0.41	Sucrose	0.84	1.03	4.66	Jellied.
43	3–4	78.30	21.70	51.32	65.53	236.49	22.70	28.99	104.60	2.26	.44	do	.75	.96	3.45	Do.
44	5–6	77.90	22.10	47.24	60.63	213.76	27.17	34.87	122.94	1.74	.57	do	.51	.66	2.32	Do.
45	7–8	77.40	22.60	41.79	53.99	184.91	31.52	40.72	139.47	1.32	.75	do	.49	.64	2.19	Negative.
46	9–10	74.30	25.70	31.79	42.78	123.69	38.37	51.64	149.30	.82	1.21	Dextrose	.44	.59	1.70	Do.
47	[2]	77.50	22.50	46.33	59.77	205.91	26.32	33.96	116.97	1.76	.57	Sucrose	.66	.86	2.96	Jellied.
				DEAD-RIPE STAGE												
48	1–2	71.00	29.00	47.99	67.62	165.48	14.92	21.02	51.45	3.21	0.31	Sucrose	1.15	1.63	3.98	Jellied.
49	3–4	80.00	20.00	55.41	69.29	277.05	18.32	22.91	91.60	3.02	.33	do	1.04	1.30	5.20	Do.
50	5–6	76.80	23.20	49.21	64.10	212.11	22.45	29.24	96.77	2.19	.46	do	.96	1.25	4.13	Do.
51	7–8	79.30	20.70	45.12	56.92	217.97	31.60	39.86	152.66	1.42	.70	do	.82	1.04	3.98	Do.
52	9–10	78.30	21.70	35.58	45.46	163.96	38.32	48.96	176.59	.92	1.08	Negative	.69	.88	3.17	Do.
53	[2]	76.70	23.30	52.08	67.93	223.51	17.62	22.98	75.62	2.95	.34	Sucrose	1.06	1.38	4.54	Do.

[1] By refractometer.
[2] Whole stalk.

163005—39——5

TABLE 4.—*Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Hodo variety of sorgo at different stages of maturity*

Sirup No.	Internodes	Total solids [1]	Water [1]	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization	Starch			
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose		On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Jellying
	Nos.	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Grams*				*Percent*	*Grams*	*Grams*	
MILK STAGE																
54	1–2	73.70	26.30	25.59	34.72	97.30	40.40	54.81	153.61	0.63	1.58	Dextrose	0.43	0.59	1.65	Negative.
55	3–4	78.00	22.00	35.58	45.61	161.72	37.17	47.65	168.95	.95	1.04	do	.29	.38	1.34	Do.
56	5–6	73.30	26.70	28.61	39.03	107.15	40.50	55.24	151.68	.70	1.41	do	.17	.24	.66	Do.
57	7–8	72.00	28.00	21.80	30.27	77.85	47.25	65.62	168.75	.46	2.17	do	.16	.23	.59	Do.
58	9–10	71.50	28.50	14.38	20.11	50.45	54.95	76.84	192.80	.26	3.82	do	.15	.22	.55	Do.
59	11–12	71.80	28.20	11.96	16.66	42.41	56.60	78.82	200.70	.21	4.73	do	.07	.10	.25	Do.
60	13–14	72.20	27.80	8.33	11.54	29.96	60.15	83.30	216.36	.14	7.22	do	.04	.06	.14	Do.
61	(2)	72.10	27.90	21.50	29.82	77.06	46.70	64.76	167.38	.46	2.17	do	.13	.19	.49	Do.
DOUGH-TO-RIPE STAGE																
62	1–2	78.20	21.80	46.78	59.83	214.58	25.15	32.17	115.36	1.86	0.54	Sucrose	0.52	0.67	2.40	Negative.
63	3–4	81.40	18.60	48.15	59.16	258.87	29.37	36.09	157.90	1.64	.61	do	.44	.55	2.40	Do.
64	5–6	77.70	22.30	39.36	50.66	176.50	34.20	44.02	153.36	1.15	.87	do	.40	.52	1.81	Do.
65	7–8	77.60	22.40	32.85	42.34	146.65	40.77	52.55	182.00	.80	1.24	Dextrose	.26	.34	1.17	Do.
66	9–10	78.90	21.10	25.44	32.25	120.56	49.85	63.19	236.25	.51	1.96	do	.21	.27	1.01	Do.
67	11–12	72.60	27.40	16.96	23.36	61.89	51.35	70.74	187.40	.33	3.03	do	.13	.19	.50	Do.
68	13–14	73.80	26.20	16.19	21.94	61.79	54.95	74.47	209.73	.29	3.39	do	.11	.16	.45	Do.
69	(2)	75.00	25.00	36.49	48.66	145.96	34.22	45.63	136.88	1.06	.94	Negative	.32	.43	1.29	Do.
DEAD-RIPE STAGE																
70	1–2	80.30	19.70	52.23	65.04	265.12	20.87	25.99	105.93	2.50	0.40	Sucrose	0.76	0.95	3.87	Negative.
71	3–4	81.30	18.70	53.44	65.72	285.77	24.02	29.54	128.44	2.22	.45	do	.54	.67	2.91	Do.
72	5–6	77.30	22.70	47.54	61.49	209.42	26.62	34.43	117.26	1.78	.56	do	.49	.64	2.17	Do.
73	7–8	77.40	22.60	42.69	55.15	188.89	31.92	41.23	141.23	1.33	.74	do	.31	.41	1.40	Do.
74	9–10	75.20	24.80	35.43	47.11	142.86	37.07	49.29	149.47	.95	1.04	Dextrose	.25	.33	1.00	Do.
75	11–12	75.10	24.90	33.16	44.15	133.17	38.97	51.88	156.50	.85	1.17	do	.20	.27	.81	Do.
76	13–14	76.00	24.00	35.28	46.41	147.00	37.65	49.53	156.87	.94	1.07	do	.18	.24	.76	Do.
77	(2)	75.30	24.70	39.36	52.26	159.35	33.05	43.89	133.80	1.19	.84	Sucrose	.42	.56	1.76	Do.

[1] By refractometer.

[2] Whole stalk.

Sucrose crystallized from 21 sirups, dextrose crystallized from 45, and 26 jellied. Only 2 sirups neither crystallized nor jellied. Eleven of the 26 sirups that jellied crystallized sucrose, 6 crystallized dextrose, and 9 jellied without crystallization.

With the exception of sirups from the Honey variety at the two later stages of maturity, when this variety dropped its seed and branched prolifically (*12*), the different sirups showed the following general tendencies.

DEXTROSE CRYSTALLIZATION

Reducing sugar content and its ratio to sucrose content were higher in sirups made from less mature sorgo and, in general, in sirups made from the lower portions of the stalk at each stage of maturity.

Dextrose crystallization was correlated with the ratio of reducing sugars to sucrose, occurring in 45 sirups in which this ratio was greater than 1. It did not occur, however, in 4 sirups in which this ratio was greater than 1.

The number of portions of the stalk giving sirups that crystallized dextrose decreased with maturity.

Dextrose crystallization occurred in sirups from all four varieties, but the extent varied.

SUCROSE CRYSTALLIZATION

Sucrose content and its ratio to reducing sugars were higher in sirups made from more mature sorgo and, in general, from the upper portions of the stalk.

Sucrose crystallization was correlated with the ratio of sucrose to reducing sugars, occurring in 21 sirups in which this ratio was greater than 1. It did not occur, however, in 7 sirups in which the ratio was greater than 1.

The number of portions of the stalk giving sirups that had ratios of sucrose to reducing sugars greater than 1 and which crystallized sucrose increased with maturity.

Sucrose crystallization occurred in sirups from all four varieties, but the extent varied in sirups from the different varieties.

STARCH

In general, the starch content was highest in sirups made from the upper internodes, and decreased progressively in sirups made from internodes toward the bottom of the stalk. As a rule, both the number of sirups that jellied and their starch content increased with maturity of the sorgo.

Jellying was correlated largely with high starch content of the sirups, although it also depended on the content of starch per 100 gm. of water. The minimum amount of starch per 100 gm. of water in sirups in which jellying occurred was 2.25 gm. (in sirup No. 29).

High starch content and the resulting jellying appear to have inhibited sucrose crystallization in five sirups (Nos. 14, 15, 20, 28, and 41) and dextrose crystallization in four (Nos. 17, 35, 36, 52).

In tables 5 and 6 the sirups are regrouped according to their crystallizing and jellying characteristics.

TABLE 5.—*Sucrose and starch content of sirups grouped according to tendency to jelly and to crystallize sucrose*

SIRUPS THAT CRYSTALLIZED SUCROSE BUT DID NOT JELLY

Sirup No.	Sucrose			Rates of sucrose to reducing sugars	Starch per 100 gm. of water	Sirup No.	Sucrose			Ratio of sucrose to reducing sugars	Starch per 100 gm. of water
	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water		
	Percent	*Grams*	*Grams*		*Grams*		*Percent*	*Grams*	*Grams*		*Grams*
8	39.97	50.40	193.09	1.22	1.91	70	52.23	65.04	265.12	2.50	3.87
45	41.79	53.99	184.91	1.32	2.19	71	53.44	65.72	285.77	2.22	2.91
62	46.78	59.83	214.58	1.86	2.40	72	47.54	61.49	209.42	1.78	2.17
63	48.15	59.16	258.87	1.64	2.40	73	42.69	55.15	188.89	1.33	1.40
64	39.36	50.66	176.50	1.15	1.81	77	39.36	52.26	159.35	1.19	1.76
SIRUPS THAT CRYSTALLIZED SUCROSE AND ALSO JELLIED											
16	38.30	49.74	166.52	1.20	3.34	49	55.41	69.29	277.05	3.02	5.20
29	40.58	52.49	178.76	1.27	2.25	50	49.21	64.10	212.11	2.19	4.13
42	52.38	63.95	289.39	2.43	4.66	51	45.12	56.92	217.97	1.42	3.98
43	51.32	65.53	236.49	2.26	3.46	53	52.08	67.93	223.51	2.95	4.54
44	47.24	60.63	213.76	1.74	2.32	47	46.33	59.77	205.91	1.76	2.96
48	47.99	67.62	165.48	3.21	3.98						
SIRUPS THAT HAD RATIOS OF SUCROSE TO REDUCING SUGARS GREATER THAN 1 BUT DID NOT CRYSTALLIZE SUCROSE											
7	34.37	46.63	130.68	1.15	1.68	28	38.76	48.75	189.07	1.31	2.56
14	41.33	54.52	170.78	1.74	3.57	41	37.40	47.58	174.76	1.05	3.74
15	44.21	57.48	191.38	1.77	3.59	69	36.49	48.66	145.96	1.06	1.29
20	37.40	50.46	144.40	1.21	2.60						

TABLE 6.—*Sucrose and starch content of sirups grouped according to tendency to jelly and to crystallize dextrose*

SIRUPS THAT CRYSTALLIZED DEXTROSE BUT DID NOT JELLY

Sirup No.	Reducing sugars as invert sugar			Ratio of reducing sugars to sucrose	Starch per 100 gm of water	Sirup No.	Reducing sugars as invert sugar			Ratio of reducing sugars to sucrose	Starch per 100 gm. of water
	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water		
	Percent	*Grams*	*Grams*		*Grams*		*Percent*	*Grams*	*Grams*		*Grams*
1	52.45	66.13	253.38	3.23	1.91	37	50.02	70.14	174.28	2.78	1.66
2	50.57	67.33	203.09	2.61	1.08	40	63.05	88.12	227.61	11.26	2.16
3	51.90	66.02	242.52	2.36	1.10	46	38.37	51.64	149.30	1.21	1.70
4	54.05	69.37	244.57	2.78	.88	54	40.40	54.81	153.61	1.58	1.65
5	65.95	86.20	280.63	11.46	.70	55	37.17	47.65	168.95	1.04	1.34
6	64.50	87.03	249.03	15.21	.27	56	40.50	55.24	151.68	1.41	.66
9	39.90	53.41	157.70	1.34	1.12	57	47.25	65.62	168.75	2.17	.59
10	48.24	66.90	172.90	2.39	.56	58	54.95	76.84	192.80	3.82	.55
11	56.11	72.12	240.81	3.37	.39	59	56.60	78.82	200.70	4.73	.25
12	56.63	72.32	260.96	3.43	.39	60	60.15	83.30	216.36	7.22	.14
13	36.80	48.74	150.20	1.10	.83	61	46.70	64.76	167.38	2.17	.49
18	43.49	58.53	170.54	1.46	1.69	65	40.77	52.55	182.00	1.24	1.17
19	40.81	56.13	149.49	1.64	1.54	66	49.85	63.19	236.25	1.96	1.01
24	57.55	78.33	217.17	4.42	1.77	67	51.35	70.74	187.40	3.03	.50
25	65.75	91.82	231.51	22.83	1.37	68	54.95	74.47	209.73	3.39	.45
26	65.30	88.96	245.49	48.01	.97	74	37.07	49.29	149.47	1.04	1.00
31	44.50	60.54	167.92	1.72	2.07	75	38.97	51.88	156.50	1.17	.81
32	54.95	71.82	233.83	3.21	2.37	76	37.65	49.53	156.87	1.07	.76
33	56.15	77.33	204.93	5.29	1.48	27	50.85	66.58	215.46	2.50	1.94
34	45.25	62.15	166.36	1.87	1.90						
SIRUPS THAT CRYSTALLIZED DEXTROSE AND ALSO JELLIED											
21	38.80	50.01	173.21	1.61	3.67	30	38.20	50.32	158.50	1.10	2.74
22	45.55	56.82	230.05	1.66	3.28	38	48.10	65.61	180.15	2.14	2.77
23	56.20	69.07	302.15	2.69	2.67	39	62.75	88.12	217.88	11.84	2.29
SIRUPS WITH RATIOS OF REDUCING SUGARS TO SUCROSE GREATER THAN 1 THAT DID NOT CRYSTALLIZE DEXTROSE BUT JELLIED											
17	35.89	47.36	147.69	1.08	2.36	36	40.50	50.62	202.50	1.26	3.00
35	40.75	52.98	176.40	1.68	2.40	52	38.32	48.96	176.59	1.08	3.17

The sirups that crystallized sucrose but did not jelly are grouped in the first part of table 5. Sirup No. 77 had the lowest sucrose content per 100 gm. of water, 159.35, and sirup No. 64 had the lowest ratio of sucrose to reducing sugar, 1.15. In four sirups, Nos. 62, 63, 70, 71, the content of starch per 100 gm. of water was greater than 2.25 gm., which as previously noted was the minimum starch content of the sirups that jellied. High ratios of sucrose to reducing sugar and a relatively high content of sucrose per 100 gm. of water characterized these particular sirups.

The sirups that crystallized sucrose and likewise jellied are grouped in the second part of table 5. The minimum ratio of sucrose to reducing sugars in this group was 1.20, and the minimum content of sucrose per 100 gm. of water was 165.48, both of which are higher than the minimum values in part 1 of this table. The higher values for these sirups were probably due to the influence of jellying on crystallization.

Seven sirups with ratios of sucrose to reducing sugars greater than 1 which did not crystallize sucrose are listed in the last part of table 5. Five of these seven sirups jellied, which probably inhibited crystallization. Thirty-nine sirups that crystallized dextrose, but did not jelly are listed in the first part of table 6. The minimum content of dextrose per 100 gm. of water was 149.30 gm., with a minimum ratio of reducing sugar to sucrose of 1.04.

Data on six sirups that crystallized dextrose and also jellied are given in the second part of table 6. In these the proportion of reducing sugars per 100 gm. of water was greater than the minimum value, 149.30, in part 1. The ratios of reducing sugars to sucrose were also greater than the minimum value, 1.04, in the first part of this table. Probably the increase was due to the influence of jellying on dextrose crystallization.

The last part of table 6 presents data on four sirups with ratios of reducing sugar to sucrose greater than the minimum in part 1. All four of these sirups jellied, as will be noted by referring to tables 1 to 4, and three exhibited a greater content of reducing sugars per 100 gm. of water than the minimum in part 1. Here, again, the effect of jellying on crystallization is apparent.

CONCLUSIONS

From data obtained in a critical examination of 67 samples of sirup made from parts of the sorgo stalk and of 10 samples made from the whole stalk, the following conclusions are drawn.

The starch content and jellying of sorgo sirups are correlated and increase with maturity of the sorgo. The upper portions of the stalk produce sirups higher in starch content. The number of parts of the stalk yielding sirups that jelly increases with maturity.

Sucrose crystallization occurs most frequently in sirups made from the upper part of the sorgo stalk. The number of parts of the sorgo stalk yielding sirups from which sucrose crystallizes increases with maturity.

Dextrose crystallization occurs most frequently in sirups made from the lower portions of the stalk. The number of portions of the stalk yielding sirups from which dextrose crystallizes decreases with maturity.

Within the range of densities applying to farm-made sorgo sirups, either sucrose or dextrose may crystallize from sirups from different parts of the same stalk. In this study it was found that when the ratio of sucrose to reducing sugars in the sirup was 1.15 or greater sucrose crystallized and when the ratio of reducing sugars to sucrose was 1.04 or greater dextrose crystallized.

As a factor in the quality of sorgo sirup, crystallization of dextrose is as important as crystallization of sucrose.

By proper selection of the parts of the stalk for milling, either sucrose or dextrose may be obtained from the sorgo plant.

Jellying and either sucrose or dextrose crystallization may occur in the same sirup.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1935. official and tentative methods of analysis . . . Ed. 4, 710 pp., illus. Washington, D. C.

(2) Berthelot, Daniel, and Trannoy, René.
1918. sur la teneur en sucre du sorgho aux divers stades de sa végétation. Compt. Rend. Acad. Sci. [Paris] 166: 824–827.

(3) Gilbee, W. A.
1858. treating saccharine fluids. Brit. Patent 655, granted Mar. 27, 1858.

(4) Janssen, Geo., McClelland, C. K., and Metzger, W. H.
1930. sap extraction of sorghum and the localization of juice and sugars in internodes of the plant. Jour. Amer. Soc. Agron. 22: 627–639.

(5) Joulie, H.
1864. études et expériences sur le sorgho à sucre considér[e] au point de vue botanique, agricole, chimique, physiologique et industriel. 200 pp., illus. Paris.

(6) Sherwood, Sidney F.
1923. starch in sorghum juice. Indus. and Engin. Chem. 15; 727–728.

(7) ———
1923. jellying of sorghum sirup. effect of malt diastase upon the filtration of sorghum juice. Indus. and Engin. Chem. 15 : 780–782, illus.

(8) Walton, C. F., Jr., and Ventre, E. K.
1937. evaporator for research on sirup manufacture. Internatl. Sugar Jour. 39 : 430–31.

(9) Wiley, H. W.
1889. record of experiments conducted by the commissioner of agriculture in the manufacture of sugar from sorghum at rio grande, new jersey; kenner, louisiana, conway springs, douglass, and sterling, kansas, 1888. U. S. Dept. Agr. Div., Chem. Bull. 20, 162 pp.

(10) ———
1892. records of experiments with sorghum in 1891. U. S. Dept. Agr., Div. Chem. Bull. 34, 132 pp., illus.

(11) Willaman, J. J., and Davison, F. R.
1924. starch in sorghum sirup. Indus. and Engin. Chem. 16 : 609–610.

(12) Ventre, Emil K., and Byall, S.
1937. distribution and variation with maturity of dissolved solids, sucrose, and titratable acidity in the sorgo stalk. Jour. Agr. Research 55 : 553–562.

THE PREPARATION AND PAINTING OF MAPLE-SUGAR-PRODUCING EQUIPMENT[1]

By C. O. WILLITS, *associate in research*, and C. J. TRESSLER, Jr., *assistant in research, Department of Chemistry, New York State Agricultural Experiment Station*

INTRODUCTION

Examinations by State and Federal authorities have disclosed the fact that maple sirup and other maple products offered in commerce sometimes contain a considerable quantity of lead. Occasionally the quantity is large enough to make the products unsafe for consumption. The maple sap as it comes from the tree has been proved by experiment to be lead-free. The lead must, therefore, be accumulated between the time the sap leaves the tree and its final processing. This limits the source of contamination to the lead-bearing surfaces of the processing equipment. These sources may be the lead-tin alloy (terneplate) coating used on some iron buckets, storage tanks, and evaporators; the lead-tin solder used in some of the joints of the metal equipment; or a paint, high in lead content, used to cover the tin-plated and wooden buckets and storage tanks to keep them from weathering.

Since all maple products produced in lead-free equipment are lead-free, a simple method of eliminating the danger of contamination would be to replace all equipment that has any lead-bearing surfaces. The cost of such a program would be prohibitive, however, and would force many maple producers out of business. A suitable material must, therefore, be found to cover up the old lead-bearing surfaces and to protect the tin-plated [2] and wooden buckets from weathering.

Such a covering may be any one of several types of paints, varnishes, lacquers, resins, etc., and the choice may be governed by many factors, the most important of which is the absence of lead. The coating should spread well when applied with a brush or as a spray. It should wear well and not chip, crack, blister, or peel when exposed to the action of sour sap, the temperature of boiling cleaning solutions, or mechanical shock unavoidable in the handling and storing of equipment.

Of the materials manufactured that might be suitable as a protective coating for maple-sirup-producing equipment, 20 were tested in the laboratory. Of these, only 8 were sufficiently low in lead (less than 100 parts per million) to be acceptable and so subject to further tests. It was found in these tests that the fact that a paint did not have its lead content printed on the label or indicated by the name was no proof that it did not contain lead. For example, 4 of the 6 aluminum paints submitted were discarded because they contained from 4 to 25 times too much lead.

[1] Received for publication December 1, 1938. Journal Paper No. 290 of the New York State Agricultural Experiment Station.

[2] Some of the tin-plated equipment has such a thin layer of tin that in a year or two it rusts through, and so must be protected.

Journal of Agricultural Research,
Washington, D. C.

Vol. 59, No. 2
July 15, 1939
Key No. N. Y. (Geneva)-15

The eight paints that passed the lead test were further examined in the laboratory and subjected as nearly as possible to all the conditions that would be encountered in the sugarbush. As a result of these tests, the paints were recommended to producers in the following order: (1) Kauri Grey Bakelite Resin Enamel, (2) Duco Aluminum, (3) Interior Tank Finish Aluminum, (4) S–W Bucket White Enamel, (5) du Pont Aluminum Paint 354–758, (6) G. L. F.[3] Lead-free Aluminum, and (7) Plicote Laboratory Aluminum Paint. Since laboratory tests often fail to show the same results that would be obtained under actual working conditions, the first three paints named, those judged best in the laboratory, were tried out in the sugarbush. The other five were not given field trials because there was not time enough to make provisions for conducting additional trials.

MATERIALS AND METHODS

Since the paints were to be tested under actual operating conditions, the cooperating producers were told only how to prepare the buckets for painting and the temperatures at which to apply the paint. The method of application was left to them. Only buckets were painted in these trials because they are undoubtedly the greatest source of lead and because they were best adapted to the experimental work. It was deemed desirable to have the selection, preparation, and painting of the buckets done by the producers in their own way, so that the results obtained would be an indication of what might be expected when the paints recommended were in general use.

Since the length of a sap season is dependent entirely upon the weather, and since as wide a range of weather conditions as possible was desired for the field trials, the paints were tried in both St. Lawrence and Cortland Counties. The tests in St. Lawrence County were made to determine the effectiveness of the paint as a coating to prevent contact of the sap with the leaded surfaces. The effectiveness was measured by the amount of lead found in the sap throughout the season. The tests in Cortland County were made only to furnish additional information on the durability of the paint.

The producers cooperating in these experiments were selected because the sirup produced in their bushes during the 1937 season had been high in lead. The buckets used in the bush of one producer were all tin-plated and well covered with a white-lead paint. Those of another producer were all painted wooden buckets, and those of a third were painted wood, tin- and terne-plated buckets.

Each producer divided the buckets that were to be painted into two groups. The buckets of one group had only the loose paint brushed out and were then washed. Those of the other group had all the old paint removed, special care being taken to remove all of it from around the seams as well as from the side walls and bottom. To do this it was necessary to use a paint remover. The one recommended was a 10-percent, or stronger, solution of commercial trisodium phosphate; and it proved entirely satisfactory both from the standpoint of ease of handling and cost. The solution is most conveniently made by adding 1 pound of the powder to 1 gallon of water, either boiling or cold. This can be poured into the buckets cold and then heated or the boiling solution can be poured into the buckets. The best and easiest way, when a large number of buckets

[3] Letters refer to Grange, Dairyman's League, and Farm Federation.

are to be cleaned, is to make up a sufficient volume of the phosphate solution in a large container, such as a scalding kettle, heat it to boiling, and dip each bucket in, then take it out and rinse. This concentration of trisodium phosphate is alkaline but not as dangerous to use as lye. The producers were warned to put their hands into the phosphate solution as little as possible and then to wash them with plenty of water. This solution when hot will remove old paint from buckets in from 2 to 15 minutes. After the paint is loosened, the phosphate solution is emptied out and the pail washed thoroughly with water to remove the last traces of the phosphate. If the phosphate is not completely removed, the new paint will blister.

The buckets were prepared for these experiments in February, and all the work of paint removal, washing, drying, and painting was done in a warm room. After the buckets were washed and rinsed, they were allowed to dry 3 to 7 days before they were painted. At least one of each type of bucket—tin, terne, and wood—from which the old paint had been entirely removed by the phosphate treatment, was painted with each of the test paints. All the buckets of the three types from which only the loose old paint had been removed were similarly treated. All of the newly painted buckets were cured for 3 weeks before they were used. At the beginning of the sap-running season, the trees were tapped by the producers and the buckets, as described in table 1, hung in each bush.

At the end of each run, a sample of sap was collected from each of the trial buckets, transferred to glass lead-free containers and taken to the laboratory. In the case of the buckets where the test paint was applied over the old paint, a composite sample was made up of aliquots taken from each of several buckets. More attention was given to the buckets in which the trial paint was applied over the old, for if satisfactory, this would be the easier way for a producer to paint his equipment.

When the sample was taken from a single bucket, 1 gallon of sap was used for analysis; when the sample was taken from several buckets, 2 gallons were used. The glass containers were made lead-free by washing first with strong alkali, then with strong nitric acid and rinsing with lead-free water. At the laboratory the sap was transferred to gallon enamel stockpots, which had likewise been treated to make them lead-free, and evaporated down to a volume that contained approximately 30 percent of sugar, which is about one-half the concentration of maple sirup. This gave a volume of nearly 250 cc. The sap was not evaporated to the density of sirup because the volume of the resulting solution would have been insufficient for making the analysis and for determining the exact moment at which to stop the evaporation when it reached a density of 32° Baumé. At no time was the sap filtered, for any cellulose filtering medium, cloth or paper, might absorb some of the lead.

When the sap had been sufficiently concentrated by evaporation, it was cooled to 20° C. and its specific gravity taken. From the specific gravity of each sample the weight of this dilute sirup which would give 10 gm. of 11-pound-per-gallon sirup was calculated. This calculated weight of the dilute sirup was used for the lead analysis. The method of analysis was a modification of the rapid colorimetric method of Perlman.[4] The results, reported in parts per million, are

[4] PERLMAN, J. L. RAPID COLORIMETRIC DETERMINATION OF LEAD IN MAPLE SIRUP. Indus and Engin. Chem., Analyt. Ed. 10: 134–135. 1938.

shown in table 1. The three maple sugarbushes in which these experiments were conducted were in locations sufficiently different to give a different number of runs of sap. After a sample was collected the bucket was emptied. The time at which the sap started to run again and the time at which the next sample was taken were recorded in order to find the length of time that the sap stood in the bucket. The daily temperature at 8 a. m., noon, and 8 p. m. were also recorded. These values are given in table 2.

TABLE 1.—*Lead content of maple sap collected during 8 runs in sap buckets of different types having different surface coatings*

Bucket type	Condition of surface [1]	Paint used [2]	Lead in run number—							
			1	2	3	4	5	6	7	8
			P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
Tin plate	Control	Old paint left on	2.00	3.38	2.30	2.55	------	3.05	3.10	6.02
	OPR	Kauri	.40	.38	.16	.10	0.30	.52	.80	------
	OP	do	.15	.40	------	.16	.82	2.05	------	2.58
	OPR	ITF	.23	.50	0	0	0	.25	.30	------
	OP	do	.04	.40	.27	.80	1.20	1.60	.82	1.27
	OPR	DA	.12	.25	------	.45	.69	1.28	1.53	1.38
	OP	do	.28	.75	.33	.35	2.19	3.49	3.64	5.10
Terneplate [3]	Control	Old paint left on	1.67	------	------	1.86	------	------	5.50	12.80
	OPR	Kauri	0	------	0	.17	------	.57	.43	------
	OP	do	0	------	0	.10	------	.32	.87	7.98
	Control	Original surface	------	------	.10	1.83	------	2.01	4.0	------
	OPR	ITF	.22	------	0	0	------	.15	1.39	.55
Wood	Control	Old paint left on	.22	------	.26	.30	------	.98	4.26	6.30
	OPR	Kauri	.13	------	.26	1.77	------	------	3.07	12.30
	OP	do	.14	------	.38	.27	------	1.00	.27	5.64
	OPR	ITF	.02	------	.23	.63	------	3.27	5.84	11.65
	OP	do	------	------	.36	.45	------	.83	------	------
	OPR	DA	------	------	.22	.10	0	.38	1.77	------
	OP	do	------	------	.07	.08	.41	.70	1.56	------

[1] OPR=old paint entirely removed; OP=over old paint.
[2] Kauri=Grey Bakelite Enamel; ITF=Interior Tank Finish Aluminum; DA=Duco Aluminum.
[3] Under terneplate there are 2 controls—(1) old paint, (2) original surface.

TABLE 2.—*Time that sap stood in buckets per given run and its temperature during this period*

Run No.	Time in buckets in—			Date	Temperature recorded			Temperature range for run		Mean temperature
	Bush A	Bush B	Bush C		8 a. m.	Noon	8 p. m.	Highest	Lowest	
	Hours	*Hours*	*Hours*		° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
1	96	------	96	Mar. 22	35	60	38	60	38	40
2	45	------	------	23	54	62	67	67	32	45
				24	32	42	------	------	------	------
3	24	8	4½	25	36	38	------	38	36	37
4	36	24	24	29	43	56	44	68	43	47.5
				30	50	68	------	------	------	------
				31	54	52	56	56	20	------
				Apr. 1	38	41	34	------	------	------
				2	31	35	------	------	------	------
5	[1] 168	[1] 168	[1] 168	4	35	20	28	------	------	37.5
				5	20	28	20	------	------	------
				6	30	35	32	------	------	------
				7	32	36	29	------	------	------
				8	34	------	------	------	------	------
				8	------	39	29	39	30	------
6	48	48	48	9	32	30	29	------	------	37.5
				11	34	------	------	------	------	------
7	48	48	48	------	------	------	50	50	35	------
				12	43	45	35	------	------	44.5
				13	35	64	------	------	------	------
				------	------	------	48	72	45	------
8	132	96	------	14	58	72	64	------	------	------
				15	53	50	50	------	------	54.5
				16	45	65	50	------	------	------
				18	54	68	------	------	------	------

[1] About 120 hours as ice and 40 hours as liquid.

EXPERIMENTAL RESULTS

A comparison of the values given in table 1 is shown in figure 1. The mean temperatures are given in table 2.

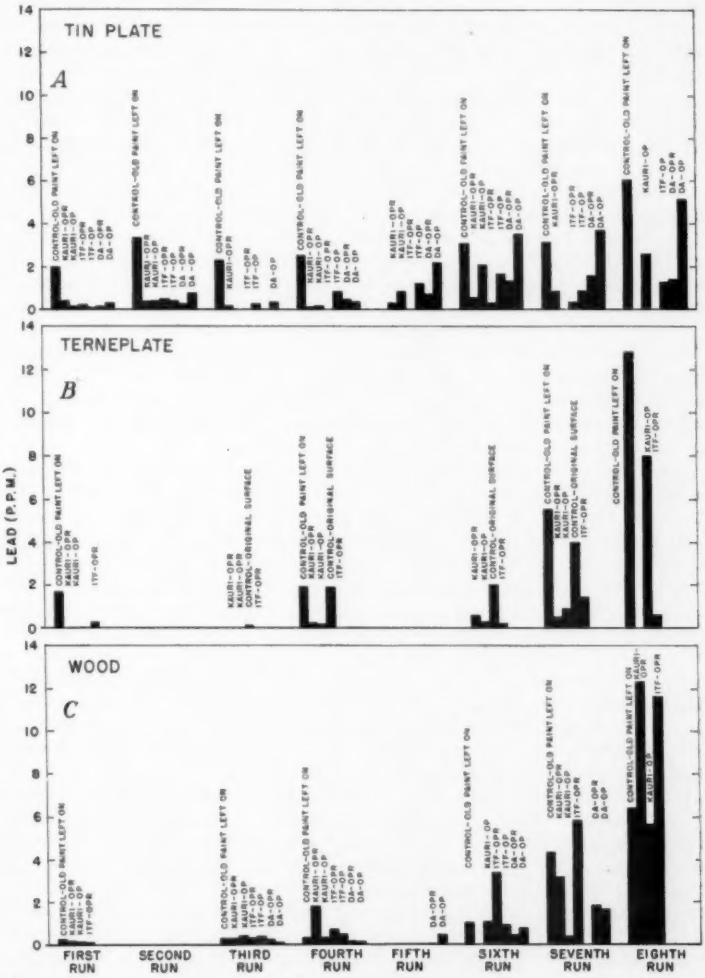

FIGURE 1.—Lead content of maple sap collected during eight successive runs in sap buckets of different types having different surface coatings: *A*, Tin-plated buckets; *B*, terneplate buckets; *C*, wooden buckets.

Comparing the lead dissolved by the sap in the control buckets where the sap was not protected from the leaded surfaces (fig. 1, *A*, *B*, *C*), the lead values are found to follow closely the mean temperature. This finding indicates that the lead-dissolving power of

the sap increases with increase in temperature; thus the higher the temperature the more lead dissolved. This no doubt is accounted for by the fact that the higher temperatures are optimum for the type of acid fermentation that occurs in the sap, and the amount of lead dissolved is directly proportional to the concentration of acid developed (the souring of the sap). During the first four runs of the sap the temperatures were low enough so that little or no fermentation took place. Consequently little acid was formed and the lead-dissolving power of the sap in all buckets was almost zero, the sap in the control buckets being nearly as low in lead as in the buckets that were protected by the trial paints. By the end of the fourth run the temperatures began to rise except during a freeze, that occurred in the middle of the fifth run. From that time on, the sap of the control buckets dissolved more lead on each successive run. The lead content of the sap, that was collected in buckets painted with the test paints remained almost constant throughout the season regardless of the test paint used, showing that the paints were about equally effective. The sap of the last run was allowed to stand in the buckets 132 and 96 hours. This is longer than a producer ever allows it to stand. Since the temperature at this time was high, the acidity of the sap was correspondingly high. This standing of the sour sap in the test buckets for so long a period subjected the paints to a severer test than they would have had under regular operating conditions. All three paints withstood this prolonged action of the warm, weak acid.

A comparison of the lead content of sap samples taken from the metal buckets, tin and terneplate, showed that the greatest protection afforded by the three paints was generally obtained when they were applied after the old paint has been entirely removed. Samples taken from the metal buckets where the test paints had been applied over the old paint contained from three to four times as much lead (fig. 1).

In the wooden buckets where the trial paints had been applied on surfaces from which the old paint had been entirely removed the amount of lead dissolved by the sap was much higher than in the buckets where the trial paints were applied over the old paint (fig. 1, *C*). This result, which is just the reverse of that obtained with the metal buckets, may have been due to the action of the paint remover on the wood fibers underlying the old paint.

All three of the paints when applied on wooden buckets, whether or not the old paint had been previously removed, withstood the effects of one sugar season very well. They neither chipped, blistered, nor peeled. This may have been due to the rigidity of the buckets and to the wood fiber surfaces upon which the paint was applied. The Grey Bakelite Resin Enamel at the end of the season showed some chipping on the bottom of some of the metal buckets, caused probably by the bulging of the bottoms during the freeze and the inability of the paint to follow the expansion of the metal because of its brittleness. No chipping of the bakelite enamel was shown in the earlier laboratory tests.

The two aluminum paints gave no evidence of chipping, blistering, or peeling from any of the buckets on which they were used, except in one bush where they were applied over old paint on metal buckets. There, toward the end of the season, a few showed blistering.

No one of the three paints had any effect upon the color, odor, or flavor of the sap. Each of the three producers expressed his preference for a different paint.

The producers cooperating in the Cortland County tests prepared and painted their buckets in a manner similar to that of the St. Lawrence County producers, but their buckets were not inspected until 1 month after the sugar season was over. The buckets had been stored away and so showed not only the effects of the use in the sugar-bush, but also that of handling at the time of storage. Fortunately, these buckets were stored by the nesting method, a method of storing that causes more abrasion than the stagger stack method. In these trials another paint, S–W Bucket White Enamel, was tried in addition to those that were tested in St. Lawrence County. No day-by-day record of temperature was kept. The season was extremely warm with only one hard freeze. This was excellent weather for testing the maple-sap bucket paints, the freeze being favorable to the chipping of the brittle paints, such as the two enamels, and the warm weather to the production of acid sap, which might cause blistering and subsequent peeling of the paint.

The results of these tests were as follows:

(1) The Bucket White Enamel was the hardest to apply, but when applied to wooden buckets it was entirely satisfactory. When applied to metal buckets, however, two out of every eight showed cracking and chipping. This paint gave no odor after 3 weeks of curing.

(2) The Kauri Grey Bakelite Resin Enamel was very easy to apply and gave much better coverage than the Bucket White. It stood up very well on the wooden buckets, but 1 out of every 10 of the metal buckets showed chipping. It still gave off a slight odor 3 weeks after painting.

(3) The Interior Tank Finish Aluminum stood up well on the wooden buckets, but when used on metal buckets 1 out of 12 showed peeling. This paint, like the Kauri, gave a slight odor after curing for 3 weeks.

(4) The Duco Aluminum was found by all the producers to be the easiest to apply. At the time of inspection, it was still entirely satisfactory, whether used on wooden or metal buckets. This paint, like the Bucket White Enamel, gave no odor after 3 weeks of curing.

The coverage, in number of buckets per quart of paint, was greatest for the aluminum paints. Next was the Kauri; the Bucket White Enamel gave the least satisfactory coverage. Eighty percent of the metal buckets in the Cortland County trials had never been painted before; where there was an old painted bucket of a given type used in the trials, there was a corresponding unpainted bucket. When there was chipping or peeling of the test paints, it occurred as often in the buckets in which the paint was applied over old paint as in those in which it was applied directly to the metal surfaces. This removes any question as to the chipping or peeling of the test paint being an aftereffect of the treatment by a paint remover. The producers noticed no color, flavor, or odor that might have been imparted to the sap or sirup by the test paint in any of these trials.

SUMMARY

The three paints, Kauri Grey Bakelite Resin Enamel, Interior Tank Finish Aluminum, and Duco Aluminum produce coatings that are very effective in keeping the lead of the leaded surfaces of sap buckets from being dissolved by the sap under normal conditions, the first two named being somewhat better in this respect than the Duco Aluminum. In commercial practice the great danger of excessive lead in maple sirup arises from the collection of sap under abnormal conditions, that is, where the sap has become very sour and is then allowed to stand in the buckets for a long time. Under these conditions the paints were, in a few cases, ineffective.

When painting metal buckets that have been painted before, the best results are obtained if the old paint is entirely removed.

A 10-percent solution of commercial trisodium phosphate, when used at or near the boiling temperature, is a very cheap and effective paint remover.

For wooden buckets that have been previously painted, better results are obtained if the new paint (any one of the approved ones), is applied over the old, any loose old paint being first removed by brushing.

If allowed to cure for at least 3 weeks after painting, none of the paints used in these experiments imparted any color, flavor, or odor to either the sap or the sirup.

U. S. GOVERNMENT PRINTING OFFICE: 1939